Foliage, shoot, and stem diseases of trees

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Foliage, Shoot, and Stem Diseases

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Preface

With our meeting in Corvallis, Oregon in 2004, scientists with special interest in diseases affecting foliage, shoots, and stems of trees began a fourth decade of gatherings to exchange information, experiences, and opinions. Every few years, freed from restrictions of time and space and other duties, the participants in the meeting of IUFRO Working Party 7.02.02 are offered a rare opportunity for direct and extended personal communication with colleagues. We are grateful to the International Union of Forestry Research Organizations for this opportunity.

Working Party 7.02.02 continues to build a new tradition of inclusiveness of the varied scientific activities of the participants. Originating from the fusion of two previous working parties with more narrow agenda, this group now engages in discussion of diseases including not only the cankers, blights, spots, and casts of traditional interest, but also rust diseases and those caused by vascular pathogens including bacteria. It is hoped that that as many forest pathologists study and attempt to mitigate damage caused by a variety of pathogens, our group will increase in attractiveness due to its appropriate breadth.

Our Corvallis meeting included 22 registrants and some additional local student attendees. Contributors from nine countries made 19 oral presentations and exhibited 12 posters, including some posters by traditional participants who found attendance at this meeting impossible. Oral and poster sessions were augmented by two day-long field tours, with observation of symptoms and signs of diseases of trees in both coastal and inland forests of the Pacific Northwest region, and expert descriptions of the biology of both pathogens and hosts.

I would like to particularly thank Greg Filip, USDA Forest Service, Portland, Oregon and Joe Holmberg, Forestry Outreach Education Office, College of Forestry, Oregon State University for their tremendously successful local arrangements including transportation, meeting facilities, refreshments, and field tours. I am also grateful to Everett Hansen, Department of Botany and Plant Pathology, Oregon State University for his substantive scientific contributions to field trips. Finally, to each participant and especially presenters, thank you for generously sharing the details of your own work, for your honest criticism and helpful suggestions, and for your good fellowship.

Contents of the abstracts and papers included in the following pages are the responsibility of the authors. Editing has been very limited, and changes were made only when the compilers have felt it necessary for the sake of style or clarity.

Glen R. Stanosz
Program Chair

Oral Presentations

Phytophthora as a foliage, shoot, and stem pathogen of forest trees

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Summary

Forest pathologists think of *Phytophthora* species as soil-borne pathogens, causing root and root crown diseases. These are water molds, after all, and we associate them with trees growing in poorly drained soils or on streamsides. The current sudden oak death epidemics in Europe and western North America are forcing a reevaluation of this dogma. *Phytophthora ramorum* is named for the dieback symptom it causes on branches of shrubs and trees in the family Ericaceae. In the forest, it is most import as a killer of oaks, causing cankers of above ground parts. There is no evidence to date of root infection in the forest. Rather, the pathogen spreads from tree to tree in rain splash driven by storm winds. Many hosts suffer only leaf blight symptoms, but *P. ramorum* may sporulate profusely on these foliar hosts without causing any significant damage to the tree. The increased inoculum, however, can be blown to other trees where it is lethal.

Phytophthora ramorum is a global threat, but it isn't alone in the forest canopy. In the course of our struggles to understand its epidemiology, we have found at least three additional unsuspected foliar phytophthoras in western forests. One is a new species, and two were recently described from European forests, as soil fungi! How many more are out there waiting for us to look up? When was the last time you used a *Phytophthora* selective medium when trying to isolate a canker fungus or foliar pathogen?

1 Introduction

Forest pathologists are familiar with several *Phytophthora* species as destructive pathogens of forest trees. Tree seedlings growing in poorly drained nursery soils are vulnerable to a diverse array of species, mostly better known as pathogens of agricultural crops (HAMM and HANSEN 1982). *Phytophthora cambivora* causes ink disease of chestnut in Europe, and basal cankers on a variety of forest trees. *Phytophthora cinnamomi*, of course, continues its destructive, omnivorous ways in forests where it has been introduced in recent centuries, from Australia to Europe and the United States. *Phytophthora lateralis* is more focused in both host selection and known geographic distribution, but is a real or potential threat to Port-Orford-cedar wherever it grows (HANSEN et al. 1999). All of these species are soil-borne pathogens, spreading by zoospores in water or transported in infested soil or roots. It is important to remember, however, that *P. infestans*, the first species described, was a foliar pathogen. The first European potato late blight epidemic spread far and fast above ground, carried on storm winds. Within the last 5 years, *P. ramorum*, another aerially transported species, has forced forest pathologists in Europe and North America to rethink the genus and the damage it can cause. In this paper, I briefly review the unique biology that makes sudden oak death such a threat, and introduce the other unexpected *Phytophthora* species with similar behavior that we have recently found. Finally, I wonder what other *Phytophthora* species await discovery, or revised understanding, in the foliage, shoots, and stems of forest trees?

2 *Phytophthora ramorum* and *P. lateralis*

Sudden oak death was first recognized in about 1995 as a distinctive and alarming disease killing coast live oak (*Quercus agrifolia*) and tanoak (*Lithocarpus densiflorus*) in mixed forests near San Francisco, CA. *Phytophthora* was recognized as the causal agent only in 2000, after other possible causes were eliminated (RIZZO et al. 2002). The pattern of disease on the landscape didn't suggest *Phytophthora*; there was no apparent association with streams or roads or other recent disturbances, although houses, and their landscaping, never seemed to be far away. Some of the hillsides where the disease was most evident in coast live oak appeared to be very dry places. The first symptoms to be recognized before the trees died were bleeding bark lesions on the main stems of the trees, with mottled necrotic phloem beneath. This symptom is reminiscent of other *Phytophthora* diseases of various trees, but these cankers appeared to be extending down the tree, not up from the roots! A number of fungi were isolated from these cankers on ordinary media, but none could be shown to incite the disease. It was only when bark pieces were plated on *Phytophthora* selective agar that the pathogen was recovered. It is perhaps worth noting that the first samples to yield *Phytophthora* were collected during a record breaking heat wave in San Francisco. Temperatures were over 100 °F that day.

It was soon recognized that the *Phytophthora* species isolated from oaks in California was identical to an unknown species earlier isolated from *Rhododrendron* in Germany (WERRES et al. 2001), and quickly named *P. ramorum*, commemorating the twig dieback symptom induced on that host. While important questions remain, progress has been rapid and we understand *P. ramorum* today as a broad-host-range, heterothallic species, producing deciduous sporangia on infected leaves and twigs, and abundant chlamydospores in infected leaf tissue. Its host range is large, and the symptoms very diverse. In addition to the girdling bole cankers associated with sudden oak death on various Fagaceae, the pathogen causes ramorum shoot dieback on a number of Ericaceae and some conifers, and ramorum leaf blight on many unrelated plant species (DAVIDSON et al. 2003). Initial infection seems to be from sporangia dispersed in rain splash and wind. In California, bay laurel (*Umbellularia californica*) is an important foliar host and source of inoculum. Although only leaf tips are colonized, sporulation is profuse. In Oregon (GOHEEN et al. 2002), initial infection apparently is in the upper crown of tanoak trees where symptoms include black, often sunken lesions on small branches (< 1 cm diameter) that still have green bark. The lesions may girdle the stem, causing a "flag" of red leaves distal to the lesion. Leaves may be the infection court. Leaves attached to these small cankers often exhibit a blackened petiole and midrib and *P. ramorum* is readily isolated. Lesions on branches larger than about 1 cm may exhibit the characteristic bleeding symptom. Sporangia form on infected leaves and stem tissues when water is available, then splash and wash down to the main bole where secondary, but lethal, cankers form. Susceptible understory plants growing immediately beneath the canopies of infected tanoaks may also be infected from the splashed and dripped inoculum. Sporangia are also evidently lofted into the wind for long distance dispersal.

The European and North American populations of *P. ramorum* are genetically distinct, and of opposite mating type, although a few 'European type' isolates have been recovered from horticultural nurseries in Oregon, Washington, and British Columbia (HANSEN et al. 2003).

Clearly the North American forest epidemic did not spread from the established European nursery and ornamental landscape epidemic, nor was Europe initially infested from North America. Both populations must have arisen elsewhere.

Phytophthora ramorum is phylogenetically closely related to *P. lateralis*, the Port-Orford-cedar pathogen established in southwestern Oregon and northern California. Both *P. lateralis* and *P. ramorum* are exotic, invasive species in North America, and must exist undetected and unsuspected in their indigenous homes, somewhere else in the world. Although both species produce chlamydospores as a prominent feature, they appear to differ in other morphological characters, and *P. lateralis* is primarily a root pathogen. Deciduous sporangia produced on the surface of solid substrates are the hallmarks of aerially disseminated *Phytophthora* species, and *P. lateralis* was first described with non-caducous (not deciduous) sporangia (TUCKER and MILBRATH 1942). But under some conditions, (poorly defined) sporangia of *P. lateralis* are deciduous (unpublished observations). Furthermore, this pathogen occasionally spreads in wind-driven rain, causing foliar infections of cedar (TRIONE and ROTH 1957). Perhaps the sporangia, not released zoospores, are the dispersal propagules in streams as well as in this rare aerial spread. Perhaps in its native habitat, *P. lateralis* is an aerial pathogen of unknown hosts.

3 Methods

Both *P. ramorum* and *P. lateralis* are subject to intensive disease management efforts in Oregon forests. This includes regular surveys to detect new infections, and intensive effectiveness monitoring of sites treated to eliminate the pathogens. In the course of these surveys a number of additional, unsuspected *Phytophthora* species have been identified. Isolations from symptomatic tissues were done on CARP selective medium (WINTON and HANSEN 2001). Soils and streams were baited with foliage baits or pear fruits. Isolates were identified to genus by gross colony appearance. *Phytophthora ramorum* and *P. lateralis* were confirmed by morphology and ITS PCR diagnosis (WINTON and HANSEN 2001). *Phytophthora* isolates other than *P. ramorum* or *P. lateralis* were initially put into water storage. Many isolates were first provisionally identified using a single strand conformational polymorphism (SSCP) protocol modified from (KONG et al. 2003). This procedure allows rapid matching of up to 96 isolates at a time with previously identified reference isolates based on chromosome mobility in a gel. Representative isolates from each electrophoretic group were then confirmed morphologically. ITS DNA sequence was obtained for isolates representing new species or species not previously found in Oregon forests.

4 Results and Discussion

One of the major surprises of the SOD research effort has been the discovery that *P. ramorum* is not the only *Phytophthora* in the upper canopy of western forests! *Phytophthora nemorosa* and *P. pseudosyringae*, are also present, causing similar symptoms, on several of the same hosts as *P. ramorum*. These two species are closely related phylogenetically to *P. ilicis*. They share key morphological features; most notably, they are homothallic, and bear caducous sporangia. *Phytophthora ilicis* is best known. It was first described as a foliar pathogen of English holly, *Ilex aquifolium*, in Oregon (BUDDENHAGEN and YOUNG 1957), and is present on holly in SW Oregon within a few miles of locations harboring the other species of the clade. Holly is not native to western North America, and it has long been speculated that its natural host must be part of the Oregon flora.

Phytophthora nemorosa was recently described (HANSEN et al. 2003) based on isolates from California and Oregon. It differs from the other species in this clade by its smaller oogonia with amphigynous antheridia and host range, as well as ITS DNA sequence. It was isolated from bole cankers on tanoak and coast live oak, and leaves of California bay. It is widespread in these forests, and locally may be more abundant than *P. ramorum*. We have also recovered it from stream water. *Phytophthora nemorosa* cannot be distinguished from *P. ramorum* in plant tissues based on symptoms, although it seems to be less aggressive in tanoak. Cankers are often smaller, and usually only single infected trees are found, in contrast to rapidly expanding clusters of diseased trees with *P. ramorum*. It is our sense that *P. nemorosa* is endemic to these western mixed evergreen forest communities.

The third western species in this clade, *P. pseudosyringae*, was also recently described, from oak forest soils in Europe (JUNG et al. 2003). In California, it is regularly encountered as a foliar pathogen of bay, producing symptoms identical to *P. nemorosa* and *P. ramorum* (RIZZO, personal communication). In Oregon, we have recovered it on several occasions from bleeding bole cankers and leaves of tanoak. It has paragynous antheridia to distinguish it from *P. ilicis* and *P. nemorosa*, but the SSCP pattern is very like *P. ilicis*. There are still more unexpected *Phytophthora* species from forest canopies awaiting characterization. Intriguingly, these include a single isolate with identical ITS DNA sequence to *P. europaea*, another European forest soil species (IVORS, personal communication).

In Britain, Clive Brasier recently encountered an undescribed species causing dieback on rhododendron and bole cankers on beech and other trees in areas where *P. ramorum* is present. The association of these new or unsuspected species with *P. ramorum* is most likely a consequence of the extra attention that forests affected by sudden oak death are receiving these days. It seems likely that some of the same, as well as other species will be found in other forest types, when people start exploring these areas with *Phytophthora* specifically in mind. It will be interesting to see if the evergreen habit exhibited by the most common hosts for these pathogens, does in fact favor aerial phytophthoras, or if this too is a coincidence of the *P. ramorum* association.

Forest soils and forest streams also harbor a diverse array of *Phytophthora* species. In the few regions that have been subject to more or less systematic sampling, from two to 10 or more species are regularly encountered. In most cases, these are not associated with acute disease symptoms, and often they are recovered from seemingly healthy forests. In European oak forests, for example, at least14 species are now known, although some are still undescribed (HANSEN and DELATOUR 1999; JUNG et al. 2002, 2003). It appears now that a similar diversity of species will be discovered in the canopies of our forests. Sometimes they are the same species, above and below ground. What sense is to be made of the common occurrence in soil and streams of *P. ramorum* and *P. nemorosa* in North America, and of *P. pseudosyringae* in soil in Europe? Or for that matter, of *P. pseudosyringae* on leaves and stems of trees in North America? I suspect that these are truly aerial pathogens, which are washed to the ground and into streams in rain water. Do they have soil saprophytic capability, or perhaps even limited potential to infect roots, or is the soil a dead end for them?

Phytophthora species are often difficult to isolate, even with selective media and special techniques. They are seldom recovered on non-selective agar media because so many true fungi are more abundant and grow faster. Because most forest pathologists don't think about water molds as foliar and twig pathogens of trees, the necessary special techniques are seldom employed when searching for causal agents. Are there dieback and leaf blight diseases currently ascribed to opportunistic Ascomycete pathogens or listed as 'cause unknown,' that are in fact incited by *Phytophthora* species? Probably. Some of the molecular diagnostic tools, such as ELISA kits, are commercially available and easy to use. They could provide a quick screen, to see if extra efforts to isolate a *Phytophthora* are warranted. The forest canopy represents unexplored territory for *Phytophthora* species. Our first forays hint at mycological riches to be discovered, and pathological dangers to protect against. Perhaps 'up there' lies the next frontier for forest pathology.

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Susceptibility of conifer shoots to infection by *Phytophthora ramorum*

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Summary

Phytophthora ramorum is the pathogen that causes sudden oak death, which was first detected on tanoak in Marin County, California in 1995. The identification of several conifers as hosts of *P. ramorum* and the increased spread of this pathogen via shipment of ornamental nursery stock has the potential to severely impact the Christmas tree, conifer nursery, and forestry industries if this pathogen spreads into major production areas. Four conifers, Douglas-fir, grand fir, yew, and coast redwood are among the naturally infected hosts that have been reported for this pathogen. There are a large numbers of different types of conifers grown as Christmas trees and in conifer nurseries in the Pacific Northwest. In an effort to better understand the potential impact this pathogen might have on the Christmas tree and conifer nursery industries, a series of inoculation studies were conducted to determine the susceptibility of foliage and shoots from 25 conifers to *P. ramorum*. Twenty of the conifers tested, including many of the important species that are used as Christmas trees, were susceptible to *P. ramorum*. Some *Abies* spp. were highly susceptible. Symptoms included needle blight, a shoot blight resulting from needle infections, and stem lesions resulting from the growth of the pathogen from infected needles into the stem. Growth stage had a significant effect on susceptibility. Needles on Douglas-fir shoots were only susceptible to infection when inoculated just after bud break. The results indicate that many different types of conifers are potentially susceptible to *P. ramorum*.

Gremmeniella abietina in Sweden - the present disease situation

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Summary

In early summer 2001, an extensive outbreak of the pathogenic fungus *Gremmeniella abietina* occurred in Sweden. Three years after the initial outbreak, the epidemic is still causing severe damage in large areas in Sweden.

At present, recommendations on sanitation thinning or clear-felling are based on experience with *G. abietina* damage on *Pinus resinosa* in North America, or with insect damages on *P. sylvestris* in Sweden. No general guidelines on management of infected slash or on regeneration after sanitation fellings have been made. In order to set management guidelines for Swedish forest owners, new studies concerning management of *P. sylvestris* stands infected by *G. abietina* have been initiated in 2001-2004.

1 Introduction

Gremmeniella abietina is frequently found in Sweden where it causes damage to the native *Pinus sylvestris* and the introduced *Pinus contorta* by killing young shoots and buds, and by forming cankers on stems and branches (HELLGREN and BARKLUND 1992; KARLMAN et al. 1994; WITZELL 2001). The fungus has repeatedly damaged artificially regenerated *P. sylvestris* stands in Sweden. The first severe epidemics occurred in nurseries during 1950s and resulted in mortality of 75 million *P. sylvestris* seedlings (BJÖRKMAN 1959; KOHH 1964). After a few unusually cold winters in the 1950s and early 1960s, strangulation damage caused by *G. abietina* was discovered in young *P. sylvestris* stands in northern Sweden (EICHE 1966). In the late 1980s, an extensive epidemic of *G. abietina* caused severe damage and mortality in young *P. contorta* plantations in northern Sweden (KARLMAN et al. 1994). In early summer 2001, an extensive outbreak of *G. abietina* occurred in middle age, 30-60 years old, *P. sylvestris* stands in Sweden.

2 Distribution of the epidemic

The geographical distribution of the epidemics has been monitored yearly 2001-2004 by the Swedish National Forest Inventory (NFI) and the Swedish National Forest Damage Inventory (NFDI). The NFI is built on stratified systematic cluster sampling with partial replacement of sample plots (RANNEBY et al. 1987). A sample of the survey tracts systematically distributed over the whole country is measured annually from the beginning of May to mid October. The NFDI is a part in a European cooperation program on monitoring forest damage (ICP Forest level I) (MÜLLER-EDZARDS et al. 1997), and is carried out annually between the middle of June and the beginning of September on a stratified selection of permanent sample plots used in the NFI (WULFF 1996).

Affected pine forests were found almost all over Sweden. Three epidemic centers were, however, distinguished (Fig. 1). The area of pine forest affected by *G. abietina* in 2001 was estimated to 328,000 ha and in 2002 to 405,000 ha. In 2003, the disease area had slightly decreased due to recovery of areas assessed as slightly diseased in 2001 and 2002 and because of sanitation felling. New areas of estimated 40 000 ha were, however, diseased in 2003. Thus, according to preliminary data, the total area affected by *G. abietina* for the three year period was assessed to 450,000 ha (WULFF et al. in preparation).

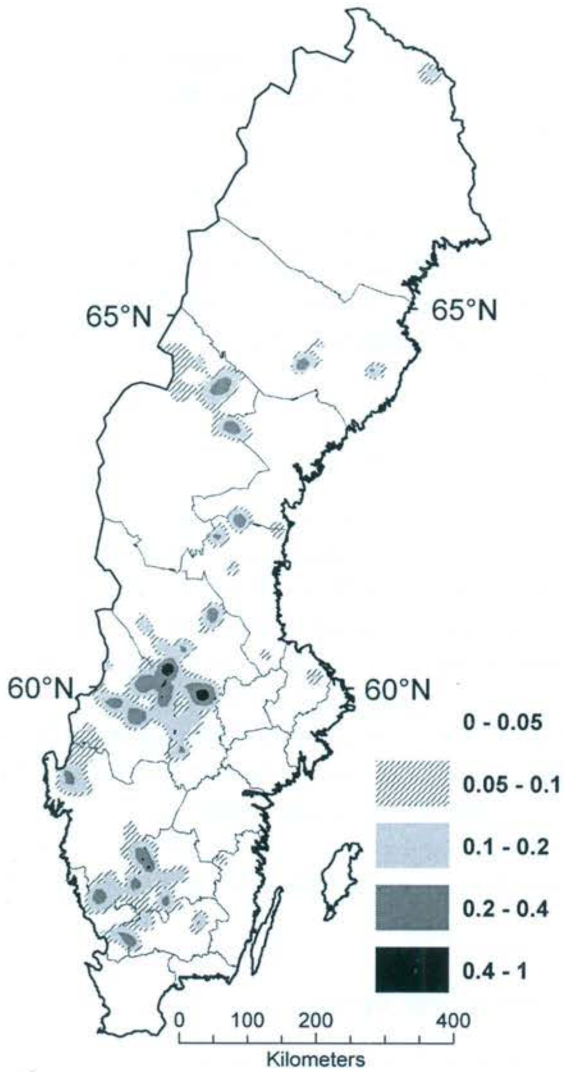

Figure 1. Disease incidence of *Gremmeniella abietina* in Sweden during the summer of 2002.

The estimated area corresponds to 5% of the Swedish pine forests. In addition, stands that have been exposed to sanitation felling before being surveyed by the NFI should be added. An underestimation of the sanitation felling area is also probable due to difficulties in defining a harvest action due to damage by *G. abietina*. This all together makes the epidemic by far the most extensive reported in Sweden.

3 Initiated research

In Sweden, current recommendations on sanitation thinning or clear-felling are built on experience with *G. abietina* on *Pinus resinosa* in North America (cf. LAFLAMME 1991) or insect attacks on *P. sylvestris* in Sweden (cf. LÅNGSTRÖM et al. 2001). No general guidelines on management of infected slash or regeneration have been made. Recently, however, several studies have been initiated in order to set management guidelines for Swedish forest owners in the future. In the following section, four of these studies are briefly presented.

3.1 Replanting after clear-felling *P. sylvestris* stands infected by *G. abietina*

The study is carried out in six sanitary clear-felled Scots pine stands in northern Sweden, all severely infected with *G. abietina*. On the treated plots the slash was removed and piled in stacks around the cleaned area. *Pinus sylvestris* indicator seedlings were planted in June and July 2002 and 2003.

Preliminary results: About 30 % of the indicator plants, on the cleaned plots as well as on the control plots, were killed by *G. abietina* after 15 months. Thus, so far the results do not indicate any reduced damage to the indicator seedlings after removal of *G. abietina* infected slash. A more thorough analysis will be made in the autumn 2004 after assessing the damage on indicator plants planted in 2003. Within the control plots the shares of infected seedlings per sub-plot vary between 4 % and 48 %. This variation will be analysed with respect to the amount of infected slash surrounding each sub-plot, which will be measured in the summer 2004.

3.2 Vitality and survival of *G. abietina* on slash after clear-felling of infected *P. sylvestris* stands

Once a month, middle-aged *P. sylvestris* trees with symptoms of recent *G. abietina* shoot blight infection are cut down and debranched. Shoots with fresh *G. abietina* fruiting bodies are marked, and grouped around each felled tree. Every consecutive month, marked shoots are randomly sampled for vitality tests of fruiting bodies and spores.

Preliminary results: Pycnidia from slash that has been on the ground for five months still have a very high germination rate, approximately 95 % (after 72 h incubation), compared to 99 % at the time of felling.

3.3 Vitality and survival of individual trees and stands with respect to infection by *G. abietina*

The study is carried out in six *P. sylvestris* stands in northern Sweden. Three of these suffer such severe damage by *G. abietina* that pre mature clear felling would be recommended according to present praxis, whereas three only have moderate damage where sanitary thinning would be considered. Within each stand plots were laid out and all trees within the plot are individually marked. The percentage of needles affected by *G. abietina* is assessed for each pine. The aim is to create an index for the relation between needle/crown loss and tree vitality and survival, and to present a recommendation on when a *G. abietina* damaged tree should be cut down.

3.4 Pruning as a tool for sanitary cleaning of infected trees and stands of *P. sylvestris*

Pruning is done mechanically with a newly developed pruning device, crane-mounted on a harvester, in three *P. sylvestris* stands damaged by *G. abietina*. The pruned plots will be compared to control plots with regard to foliage damage. Cut branches will be left on the ground and inspected once a month regarding fruiting bodies. Shoots will be randomly sampled for vitality tests in accordance with studies earlier mentioned.

The results of these studies will be evaluated during 2004-2006 and provide significant knowledge on management of *G. abietina* damaged *P. sylvestris* stands. This will be of great importance for setting management guidelines for Fennoscandian forest owners in the future.

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The role of winter hardening in *Pinus sylvestris* L.-*Gremmeniella abietina* (Lagerb.) Morelet plant-pathogen interactions

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Summary

The ascomyceteous pathogen *Gremmeniella abietina* (Lagerb.) Morelet is the causal agent of shoot and twig dieback in several genera of conifers. It is favoured by cold, wet summers and mild winters. *Gremmeniella abietina* infects the top shoots of its host in the summer, and stays there as a latent infection until winter, when it starts to grow in the innerbark and into the wood. Preparing for winter, the trees lower their growth and metabolistic activity. During this winter-hardening process, the trees also build up a certain degree of frost tolerance by withdrawal of water from the tissues. Thus, the freezing point in the cells is lowered and lytic leakage is avoided. Lowering metabolism, the trees also lower their guard to pathogens. Previous studies have shown that *G. abietina* is able to take advantage of its host tree's weakened defense mechanisms. It has also been hypothesised that *G. abietina* needs at least 44 conducive days of mild winter weather with temperatures near zero °C in order to be able to break its latency.

In our study, we pretreated 2-year-old pine seedlings in three separate regimes. The first third of the seedlings were pretreatred and let to winter-harden in an outdoor regime. Another third were winter-hardened in a controlled regime in a climate chamber, where the night length was gradually prolonged and day length equally shortened, and the temperature gradually lowered over time. The remaining seedlings were placed in a stable regime in a climate chamber at 12 h night/12 h day per 24 h to prevent them from winter-harden. During the course of the experiment seedlings were taken from the pretreatment regimes and subsequently inoculated with one of four isolates of *G. abietina* or sterile agar (control). The inoculated seedlings were incubated in a climate chamber for 60 days at 5°C, 90% relative humidity and 16 h night/8 h day, which corresponds to mild Swedish winter conditions. After the 60 day period, results were read. Disease incidence was defined as no. of seedlings expressing disease symptoms in the inoculated shoot (i.e., visible necrosis). Disease severity was estimated by measuring the necrosis length in mm.

Seedlings winter-hardened in the climate chamber showed a significantly lower degree of disease incidence and severity than the seedlings winter-hardened outdoors. Instead they showed about the same disease incidence and severity as the seedlings pretreated in the stable regime. This implies that the winter-hardening process itself doesn't predispose the host tree for *G. abietina* infection. Nor can the conducive days theory solely explain the growth of *G. abietina* in the host during winter. We believe that the tree has to be predisposed to infection, and that they become predisposed when subjected to sudden large temperature drops during or after winter-hardening.

Can we identify *Gremmeniella* types according to ascospore morphology?

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Summary

Twenty-one type A and twelve type B samples with ripe apothecia were collected from Sweden and Finland. The ascospores were released on objective slides. The slides were stained with anilin blue. Then the ascospores were measured with 400 X magnification with image analyser program (Leica QWin). The measured variables were length, width, perimeter and roundness of ascospores. The distributions of these variables were overlapping. The type A ascospores were shorter (mean 15.9 µm) than type B ascospores (mean 17.2 µm). Type A ascospores were also rounder (1.9) than type B ascospores (2.2). These differences are too minute to use as a morphological character for describing types as new taxonomic species. Round-pointed and acute ascospores were found even in same apothecium in different asci. The monospore isolates of both ascospore shapes were analysed for sequences of a multiallelic SCAR-marker. According to this analysis round-pointed and acute ascospores can have the same allele in this locus. In ten analysed monospore isolates it was found two alleles of SCAR-marker. This means that ten monospore cultures were originated from two parents. The parents could not have different alleles of ascospore shape, because different shapes of ascospores were found in different asci.

Key words: *Gremmeniella abietina*, ascospores, ascospore morphology

Taxonomic diversity of viruses inhabiting *Gremmeniella abietina* in Finland

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Summary

Double stranded RNA (dsRNA) viruses of *Gremmeniella abietina* were first observed in 1997, when samples of *G. abietina* var. *abietina* type A were observed to host two common dsRNA patterns, composed of one and three segments, respectively. In year 2001 a third dsRNA pattern was observed. The nucleotide sequences of the cDNA of all these three dsRNA patterns were determined, and the sequences suggested that they encoded viruses belonging to genera *Totivirus*, *Partitivirus* and *Mitovirus*. The Partitivirus was the most frequently observed one, and Totiviruses occurred mostly in isolates from asymptomatic tissues. The Mitovirus was observed in only one isolate. In year 2002 a single isolate was tested for the occurrence of dsRNA, and found to include altogether five dsRNA segments. The sequences of these segments indicated that the isolate was inhabited by members of all three different virus families known to occur in *G. abietina* type A. The three viruses occurred in different compartments within the cell, as shown by an equilibrium ultracentrifugation experiment, and were efficiently transferred to conidiospores. In year 2003 a new sampling to find viruses in *G. abietina* was conducted in four locations. This time the most commonly observed virus was a Mitovirus; whereas only few Totiviruses or Partitiviruses were observed. Therefore a considerable change in the virus community parasitising *G. abietina* type A seemed to have occurred in Finland in six years. Few isolates of *G. abietina* type B were also tested for the occurrence of dsRNA, and found to include two dsRNA-patterns. Preliminary sequences suggest that one of the patterns belongs to a virus belonging to genus *Mitovirus*, whereas the other one is unrelated to viruses found in *G. abietina* type A.

Phaeocryptopus gaeumannii and Swiss needle cast disease in Oregon

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Summary

Despite sporadic outbreaks of Swiss needle cast disease (SNC), *Phaeocryptopus gaeumannii* has been considered an inconsequential forest pathogen in western North America. Increasing SNC severity observed in Douglas-fir plantations along the Oregon coast since about 1990 has prompted renewed interest in the biology of the pathogen and factors influencing disease severity. Coordinated research on the systematics, population genetics, and infection biology of *P. gaeumannii*, and on epidemiology and management of SNC has contributed to a better understanding of how an insignificant foliar parasite has become an important forest health problem in the Pacific Northwest. Phylogenetic analyses of nrSSU, nrLSU, and nrITS show *P. gaeumannii* to be more distantly related to *P. nudus* (type species of *Phaocryptopus*) than to other members of Dothidiales. *Phaeocryptopus gaeumannii* shares the greatest degree of sequence homology with species in the genus *Mycosphaerella*. The need for changes in nomenclature and classification of *P. gaeumannii* is recognized. In the Pacific Northwest, and in particular in Coastal Oregon, *P. gaeumannii* exists as two distinct, reproductively isolated, sympatric genetic lineages or sibling species. Results of SSCP analysis for one mitochondrial and four nuclear genes, and for 10 polymorphic microsatellite loci indicate that the genetic diversity is low in populations from outside the Pacific Northwest, where Douglas-fir has been grown as an exotic species, and high in populations from the Pacific Northwest, consistent with the presumption that *P. gaeumannii* is native to the region. Lineage 1 has nearly worldwide distribution, occurring throughout much of the Pacific Northwest as well as in exotic locations that have historical reports of disease. Lineage 2 is restricted to Oregon's coastal forests. Results of seedling inoculation experiments suggest that differences in virulence between the two lineages exist: isolates of Lineage 1 caused greater needle loss on seedlings one year after inoculation than comparable inoculations with Lineage 2. Site-specific microclimate also influences disease severity. A strong relationship was found between winter (Dec-Feb) mean daily temperature and disease severity in forest plantations in Coastal Oregon. This led to the development of a disease prediction model employing GIS-linked climate models for western Oregon. Management approaches for control of Swiss needle cast in forest plantations are limited. Annual aerial fungicide applications over several consecutive years increase needle retention and increase growth in diseased stands, but disease levels return to pretreatment levels withing 2-3 years following cessation of annual fungicide treatment.

Alterations in Douglas-fir crown structure, morphology, and dynamics imposed by the Swiss needle cast disease in the Oregon Coast Range

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Summary

Plants respond to defoliation in many different and complex ways, depending on their growth habit and form as well as the extent and duration of the defoliation. Tree crowns have been shown to be quite sensitive to disturbances such as defoliation. However, quantitative relationships have rarely been developed, making the true biological meaning of crown condition assessments quite difficult to decipher. The sudden emergence of Swiss needle cast (SNC) in the Oregon Coast Range prompted investigation of the response of Douglas-fir crown structure, crown morphology, and foliage dynamics to extended defoliation. Using data from permanent plots and 82 destructively sampled trees, hypotheses regarding the response of trees to defoliation were tested with linear and nonlinear models. Responses of crowns were investigated at multiple levels including the needle, branch, tree, and stand scales. Analysis indicated that the defoliation due to SNC caused significant changes at all levels, complicating efforts to accurately predict growth responses to the disease. Important alterations included: reductions in needle size and mass; altered branch allocation and growth; shifts in the foliage age class structure and vertical distribution of leaf area; modified crown size and shape; and improved stand growth efficiency. While crown condition can be highly variable and difficult to assess, it is an important measure that needs to be to be continually examined and incorporated into current forest surveys given the significant ecophysiological implications of this study.

Incidence of *Gremmeniella abietina*, *Rhabdocline pseudotsugae*, *Chrysomyxa abietis* and *Phyllosticta concentrica* in Christmas tree cultures and gardens in Hungary

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Summary

Needle and shoot pathogens uncommon in Hungary were observed in recent years in Christmas tree cultures, gardens and arboretums. *Gremmeniella abietina* occurred on spruce (*Picea abies*) causing yellowing of the second year old needles. The multiseptate, large conidia suggest the alpine biotype of the fungus despite of its occurrence at low altitudes a.s.l. *Rhabdocline pseudotsugae* caused severe needle cast on Douglas fir. *Chrysomyxa abietis* and *Sclerophoma pythiophila* were observed causing occasionally needle and shoot diseases on Colorado blue spruce (*Picea pungens*). *Phyllosticta concentrica* was observed on English yew (*Taxus baccata*) associated with conspicuous shoot blight. These needle and shoot pathogens do not appear usually in forest conditions in Hungary. Their occasional incidence in gardens and Christmas tree plantations could be of interest regarding the European spread and hosts of the pathogens. This is the first report of *G. abietina* on spruce and *Chrysomyxa abietis* on Colorado blue spruce in Hungary.

Key words: *Gremmeniella abietina, Rhabdocline pseudotsugae, Phyllosticta concentrica, Chrysomyxa abietis, Sclerophoma pythiophila*, needle and shoot diseases, spruce, Douglas-fir, English yew

1 Introduction

The forests in Hungary are composed mainly by broad leaved trees. Conifers represented mainly by planted Scots and Austrian pine stands cover about 15% of the forest surface. Spruce and fir have small plots only at the limit of their natural range, as well as Douglas-fir originally from Pacific North America. *Sphaeropsis sapinea* (Desm.) Dyko et Sutton and *Mycosphaerella pini* E. Rostrup ap. Munk are common needle and shoot pathogens of pines in forestry conditions. *Lophodermium seditiosum* Minter, Staley et Millar is an important pathogen in nurseries. Some needle pathogens common in conifer forests in other parts of Europe and in North America appear in Hungary sporadically only, mostly in gardens and Christmas tree cultures.

2 Material and Methods

Cases of incidence of needle and shoot diseases were investigated in Christmas tree cultures and gardens. The severity of the diseases was estimated, the pathogens identified, occasionally isolated, and their pathogenicity tested by infection of seedlings.

3 Result and Discussion

Selected cases of needle and shoot diseases observed in Christmas tree cultures and gardens are shown in the following.

3.1 *Gremmeniella abietina* (Lagerb.) Morelet

In June 2003 yellowing of second-year-old needles of spruce (*Picea abies*) was observed in Christmas tree plantations in Western Hungary at altitude of 150-200 m a.s.l. Pycnidia of the conidial state of *G. abietina* were observed on the second year old living twigs. The conidia had mostly 7 septa and measured 45-95 x 2.5-5 μm.

Gremmeniella abietina, the causal agent of Scleroderris canker, is distributed in Europe, North America, and Asia. Two varieties of the fungus were distinguished based on the morphology of the anamorph: *Brunchorstia pinea* var. *typica* with mostly 3 septate and 19-55 x 2.5-4 μm conidia, distributed in North America, Europe and Asia on *Pinus, Picea, Abies, Larix*, and *B. pinea* var. *cembrae* characterized by 5-7 septate and up to 73 μm long conidia, occurring in the Alps at high altitudes on *Pinus cembra* (MORELET 1980). Two varieties of the fungus were distinguished by morphology and protein electrophoresis patterns: *Gremmeniella abietina* var. *abietina* (European, North American, and Asian races), and *Gremmeniella abietina* var. *balsamea* occurring on *Picea* and *Abies* in Canada (PETRINI et al. 1989). The European isolates of the fungus were grouped then in three amplitypes based on their distinct DNA amplification profiles correlating with ecological aspects: a cold adapted amplitype occurring on *Pinus cembra, P. mugo, P. sylvestris* and *Larix lyalli* at high altitudes in the Alps (alpine amplitype), apparently corresponding *to G. abietina* var. *cembrae*, a northern amplitype found above 66 degrees latitude on *P. sylvestris* and planted *P. contorta* (finnoscandian amplitype), and the European amplitype widely distributed in Europe (HAMELIN et al. 1996).

Our specimen possessing mostly 7 septate, large conidia seems morphologically to belong to the alpine type, despite of its occurrence at low altitudes and in a host (spruce) not registered for this type before. Moreover one of our herbarium specimens of the fungus originated from the Alps at high altitudes in *Pinus cembra* has three septate and 25-55 x 2.5-4 μm sized conidia characteristic for the European type. These facts indicate that the distribution and hosts of the types of *G. abietina* in Europe are still not known entirely.

This is the first certain record of *Gremmeniella abietina* in Hungary. The pathogen did not seem to be lethal for spruce. The trees survived the infection, suffering partial needle cast and loss of some thin twigs.

3.2 *Rhabdocline pseudotsugae* H. Sydow

The needle pathogen *Rhabdocline pseudotsugae* has been known in Hungary for several years occurring sporadically in the Douglas-fir stands and Christmas tree cultures in western part of the country (SZABÓ 2002). In April 2003 severe attack of this fungus was observed in a Christmas tree culture near Sopron. The maturation of the apothecia and ascospore release was on during May, followed by casting of infected needles.

The grade of the disease was estimated as 2.3 using a scale of 4 stages. Chemical control was applied (flusilasol, karbendazim, mancozeb) two times during the spore release period. In spring 2004 the disease appeared in a minor grade of mean 0.6.

3.3 *Chrysomyxa abietis* (Wallr.) Unger

In May 2002 needle rust was observed on Colorado blue spruce (*Picea pungens* Engelm.) in an arboretum in western Hungary. The needles of the preceding year showed yellowing symptoms and orange telia of *Chrysomyxa abietis* were observed on their under surface. Teliospores arranged in short chains measured 25-55 x 7.5-12.5 μm, meanly 35.75 x 11.37 μm. *Chrysomyxa weirii* damaging *P. pungens* in North America differs by its smaller teliospores (GAUMANN 1959).

This is the first record of *C. abietis* on *P. pungens* in Hungary. The *Chrysomyxa* species are not frequent in Hungary even on the common spruce. Most of the previous data of their occurrence refer to the mountain territories outside of the actual borders of the country.

Several occasions of tip drying were observed last years in Colorado blue spruce in Christmas tree cultures. Some weak pathogenic fungi were identified on the drying twigs, most frequently *Sclerophoma pythiophila* (Corda) Höhn. *Rhizosphaera kalkhoffii* Bubák and *Tiarosporella parca* (Berk. et Broom) Whitney were recorded less frequently. Results of a pathogenicity test carried out by inoculating young shoots of seedlings with mycelium of *S. pythiophila* were negative. We concluded that this fungus causes disease to the hosts weakened by other factors only. The observed symptoms could be associated primarily with dry and warm weather of the last summers.

3.4 *Phyllosticta concentrica* Sacc.

Unusual shoot blight was observed on English yew in spring 2003 in several gardens. The young shoots turned light brown before their complete maturation. Pycnidia of *Phyllosticta concentrica* Sacc. appeared in the necrotized tissues. This fungus is a true *Phyllosticta* with spherical pycnidia and oval conidia bearing mucous appendage on their apex (VAN DER AA 1973). *Phyllosticta concentrica* was known before as a weak pathogen of yew colonizing the old needles and the internal, shaded twigs, causing their cast mostly in years with wet weather in spring (SZABÓ 1997). Appearance of shoot blight is thought to be primarily a consequence of the weather conditions of the spring 2003, early warm followed by lasting cool and extremely dry weather.

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Needle cast on nordmann fir in Norwegian Christmas tree plantations

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Summary

Nordmann fir/Caucasian fir (*Abies nordmanniana*) is the dominant Christmas tree species in Norway. In many plantations there are problems with heavy needle cast. The fungus *Rhizosphaera kalkhoffii* is often detected in dying needles on lower branches. Another serious needle cast is green, current season needles falling off during the winter, both on newly harvested trees and in the field. This needle cast is mainly located on the top shoot and the upper branches. Two fungi are associated with this symptom; *Thysanophora penicillioides* and a not yet identified synnematous, dematiaceous hyphomycete. The latter was found in April 2004 on up to 73% of the needles that fell off in a field when the trees were hand shaken.

1 Introduction

In Norwegian nordmann fir plantations for Christmas tree production, three different species of fungi associated with the stomata have been found on trees with heavy needle cast: *Rhizosphaera kalkhoffii* Bubàk, *Thysanophora penicillioides* (Roum.) Kendrick, and an unidentified, synnematous, dematiaceous hyphomycete. *Rhizosphaera kalkhoffii* is found on discoloured needles, and the two latter fungi are found on green needles that drop during the winter.

Rhizosphaera kalkhoffii is a coelomycete that has pycnidia growing in rows along the stomata. The other two fungi are hyphomycetes with relatively long conidiophores. They are also located along the stomata rows. *Rhizosphaera kalkhoffii* has been reported on a wide variety of conifer hosts from around the world, and it is common in Europe (MAANEN and GOUBIÈRE 1997). From Canada, *R. kalkhoffii* is reported on *Abies*, *Pinus*, and *Pseudotsuga* (FUNK 1985). In the Pacific Northwest it causes significant disease on *Picea* (BRYTHER and CHASTAGNER 1997).

According to KENDRICK (1961), *Thysanophora penicillioides* was only observed on needles after they had dropped to the forest floor, and thus the fungus was considered a saprophyte. Also the work by VAN MAANEN and GOURBIÈRE (1997) describes the fungus on decaying conifer needles (litter samples) in a European fungal distribution study. *Thysanophora penicillioides* is also described as a saprophyte by ELLIS and ELLIS (1997). So far the only literature cited that describes *T. penicillioides* in living needles is from a needle entophyte survey in Finland on Norway spruce. In that investigation, *T. penicillioides* was isolated occasionally from green needles taken from trees (MOLLER and HALLAKSELA 1998).

The shedding of green needles during the winter is also reported on nordmann fir in Denmark, where it is most prominent from December to February, but some winters are said to be worse than others. One theory from Denmark suggested that a sudden drop in temperature before the plants were fully hardened in the fall resulted in needle cast (LYHR 1994).

This paper presents the needle cast problem on nordmann fir as it appears in Norway. It is previously described in fact sheets for growers (TALGØ and STENSVAND 2003a; TALGØ and STENSVAND 2003b).

2 Materials and methods

2.1 Field observations

In March 2004, heavy needle cast was reported in a nordmann fir field on the west coast of Norway (Rogaland county). The field was inspected and samples taken (see "Samples investigated"). Based upon the results from the samples taken in March 2004, a survey was carried out in May 2004 to establish the percentage of damaged nordmann fir trees. Number of trees with needle cast was recorded in four different plots at one location and two plots at another location, both in Rogaland county. History of trees with needle cast was collected from the growers.

2.2 Samples investigated

A total of 106 samples from damaged nordmann fir in nurseries and production fields were tested for presence of foliage diseases in the laboratory at the Norwegian Crop Research Institute during 2001-2003. The needles and shoots were incubated in saturated air at room temperature and examined for fungal growth. Sample size varied from a few needles to whole 4-5 year old trees. Depending on the condition of the samples, the length of the incubation period varied. Normally 2-5 days gave satisfactory fungal growth, but samples were sometimes kept longer.

In March 2004, samples were collected from four trees in one plantation with heavy needle cast. The trees were vigorously shaken for a few (2-3) seconds, and needles that dropped were collected on a sheet spread out on the ground under the trees to avoid contact with the plantation floor. The needles easily dropped when the branches were shaken. One hundred needles from each tree were incubated in saturated air at room temperature. The needles were oriented with the stomata facing upwards. On some needles, fungal growth was visible already the same day the needles were incubated. The final assessments were carried out 10 days later.

2.3 Pathogenicity test

To fulfil Koch's postulate, a pathogenicity test was carried out to confirm the parasitic ability of *T. penicillioides*. One ml of sterile, distilled water was added to a sporulating PDA-culture (90 mm Petri dish). Twenty nordmann fir container plants were inoculated with *T. penicillioides*. The trees were inoculated by dipping a cotton tipped sterile applicator in the spore suspension and carefully applying it to the soft, new shoots. Ten of the plants were wounded by applying Carborundum prior to the inoculation. The room temperature was kept at 20°C and the plants were given 16 hours daylight. The test started on 12 May 2004. A polyethylene bag was kept over each plant until 1 June. After the bags were removed, the relative humidity was kept at approximately 80%. Similar pathogenicity tests have not yet been carried out for *R. kalkhoffii* and the unknown hyphomycete.

3 Results

3.1 Field observations

The number of affected trees varied considerably from field to field, but 2-3% was common. The most extreme case was observed on young trees. The trees had only been in the field for one year, and 230 out of 860 trees (27%) had visible needle cast.

Growers reported that the same trees had problems with needle cast year after year. In addition, new cases showed up every year on all age classes.

3.2 Samples tested

From the 106 samples of nordmann fir that were incubated in the period 2001-2003, *R. kalkhoffii* was present on 25 % and *T. penicillioides* on 30 % of the samples. *Thysanophora penicillioides* and *R. kalkhoffii* were often found in the same samples, but only rarely observed to colonise the same needles. *Thysanophora penicillioides* was frequently observed sporulating from incubated material taken from green needles still attached to branches located towards the top of the trees. *Rhizosphaera kalkhoffii* was found on needles from all over the trees, but was more abundant on lower branches, where infected needles turned yellow and finally brown before they were shed.

The results from the incubation tests of needles collected in March 2004 are presented in the table below. On many needles, the majority of the stomata were completely covered by fungal growth.

	Tree I	Tree II	Tree III	Tree IV
Rhizosphaera kalkhoffii	1	1	1	23
Thysanophora penicillioides	0	1	5	5
Unidentified fungus	73	5	19	33
T. penicillioides and unidentified fungus on the same needle	0	0	1	2
Total	74	7	26	63

Table 1. Number of needles (out of 100 needles per tree) from *Abies nordmanniana* that were colonised by either *Thysanophora penicillioides*, *Rhizosphaera kalkhoffii*, or an unidentified hyphomycetous fungus. Sampling took place in March 2004, and the samples were incubated in saturated air for 10 days at room temperature.

As shown in Table 1, the unidentified hyphomycete colonised 73% of the incubated needles from one of the trees. No culture is obtained of this fungus, but glass slides, photos and herbarium material are available.

3.3 Pathogenicity test

By the deadline of this article no symptoms were visible on nordmann fir plants inoculated with *T. penicillioides*.

4 Discussion

Both *T. penicillioides* and *R. kalkhoffii* have been found previously on nursery stock in Norway (V. TALGØ, unpublished data), so the plants in the field where 27% had needle cast might well have been infected by the time of transplanting.

From what was observed by growers in Norway, the theory that needle cast is caused by unsatisfactory hardening conditions in the fall does not apply, because the same trees were seen to loose needles year after year despite years when conditions for winter hardening were good. A possibility is that the trees facing problems were not of the right provenance, because seed sources are known to be of mixed quality. Sometimes the needle cast trees were grouped together, but they were more commonly spread out in the field in a random pattern. This suggests that we are not dealing with something epidemic, but more individual plants that for some reason are more susceptible than others, which in general is often the case with fungal diseases.

The findings of *T. penicillioides* on green needles still attached to trees strongly indicate that this fungus might be a parasitic organism. Interestingly, some samples of nordmann fir that were tested after they were harvested in December 2003 because of heavy needle cast, had needles still attached to the branches that were completely covered with *T. penicillioides* after incubation. That may indicate that *T. penicillioides* plays a role in post-harvest needle retention.

It might be possible that the needles drop because the gas exchange is restricted by the fungus physically clogging up the stomata, and thereby reducing the photosynthesis.

Few of the needles still attached to the trees that were tested in March 2004 (Table 1) were attacked by *T. penicillioides*. However, by March many needles had already dropped, and the findings on samples from December 2003 indicated that needles with *T. penicillioides* were very loosely attached to the trees already in the fall.

The unidentified fungus was believed isolated in April 2004, but sequencing of the culture at Central Science Laboratory in England revealed that it was the coelomycete *Hormonema dematioides* Lagerh. & Melin, the conidial state of *Sydowia polyspora* (Bref. & Tav.) Müller (99% match). In future attempts to isolate this fungus, water agar will be used instead of PDA, because *H. dematioides* is known to be fast growing on rich media and thereby able to cover up more slow growing fungi. *Sydowia polyspora* is known to give complete defoliation of pine in association with pine needle midge attacks, but is not reported to damage fir (SUTTON and WATERSTON 1970).

Both *R. kalkhoffii* and *T. penicillioides* seem to be widely spread in Norway, because both fungi are found on many locations and on a number of different fir (*Abies*) and spruce (*Picea*) species. Besides on nordmann fir, *R. kalkhoffii* has been found on Turkish fir (*A. bornmuelleriana*), grand fir (*A. grandis*), Korean fir (*A. koreana*), subalpine fir (*A.*

lasiocarpa), cork fir (*A. lasiocarpa* var. *arizonica*), noble fir (*A. procera*), Norway spruce (*P. abies*), and engelman spruce (*P. engelmannii*) (TALGØ and STENSVAND 2003a). Except for Turkish fir, *T. penicillioides* has been found on the same hosts as *R. kalkhoffii* in Norway, and furthermore it has been found on Siberian fir (*Abies sibirica*) (TALGØ and STENSVAND 2003b).

In Norway, growers refer to the trees that loose green needles during the winter as "skeletons", because in severe cases they become nearly bare, but they do not die. As the picture (Fig. 1) shows, new buds break in May. In Denmark, growers are advised to cut down "skeletons". That might well be the only recommendable advice, but since large numbers (up to 27% in this survey) of the trees in Norway may be affected, it is considered well worth to investigate this disease complex further. The work will start in the fall of 2004 and will include isolation, identification and pathogencity tests with the unidentified hyphomycete, a post-harvest survey of trees to see if any of these fungi are involved when trees have bad needle retention, and finally, a number of production fields will be investigated during winter to get a better understanding of why some trees are shedding green needles.

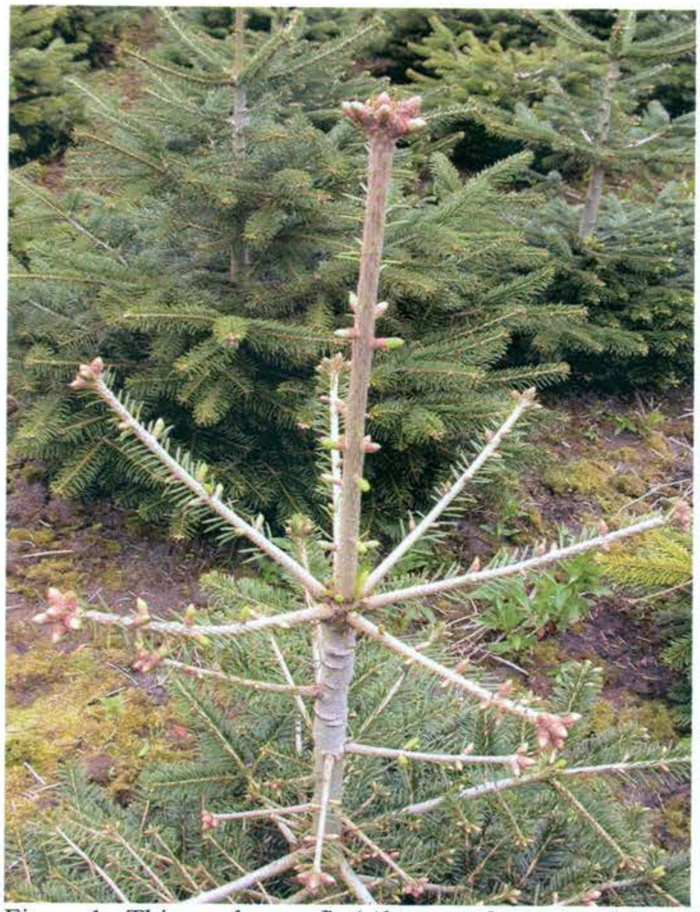

Figure 1. This nordmann fir (*Abies nordmanniana*) in a Christmas tree field on the west coast of Norway had lost many needles during the winter 2003/2004. The picture was taken 13 May 2004, by the time when new buds were about to break. Photo: V. Talgø.

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Physiological and histological evidence for non-host resistance within the *Melampsora/Populus* pathosystem

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Summary

Reactions to rust infections have been classified as "host" or "non-host" based on certain aspects of the infection process. Throughout North America the native *Populus* spp. are host to one or more of the indigenous *Melampsora* spp. We investigated the nature of resistance in compatible and incompatible combinations by electron microscopy (EM) and biochemical analysis. *Populus trichocarpa* and *P. deltoides* were inoculated with *M. occidentalis* and *M. medusae*. When examined by EM, hyphal ramification was extensive in all treatments. In the compatible combination, multiple haustoria were already present at 48 hr postinoculation and wall appositions had formed by 144 hr. In the incompatible combination colonization ceased shortly after the appearance of the first haustorium and by 96 hr haustoria were either encased within the cell or beginning to degerate. Total peroxidase content was determined at 24 - 120 hr postinoculation. Isozyme patterns were similar for both the compatible and incompatible interactions, but peroxidase activity was enhanced in the incompatible interaction. Both these responses characterize non-host resistance. Since interspecific hybrids of *Populus* can be made, we propose the *Melampsora / Populus* pathosystem as an ideal vehicle to investigate the genetics of non-host resistance.

The infection behaviour of four shoot pathogens of pine: experience in Britain

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Summary

Contrasting infection behaviour is shown by the four pine shoot pathogens *Sphaeropsis sapinea, Brunchorstia pinea (Gremmeniella abietina), Ramichloridium pini,* and *Cenangium ferruginosum*. With *Sphaeropsis sapinea* on pines such as *Pinus nigra* ssp. *austriaca*, infection of the developing shoot is quickly followed by host invasion with the result that the shoot is partially or entirely killed before it completes its growth. With *B. pinea* on *P. nigra* ssp. *laricio* the developing shoot is susceptible to infection in mid-summer, but host invasion only takes place during the following dormant season. With *R. pini*, a pathogen of *P. contorta* not known in its native habitat, the process is similar. *Cenangium ferruginosum* may behave in a somewhat similar way on *P. nigra* ssp *laricio* and *P. sylvestris,* with the constraint that host invasion only occurs under unusual dormant season conditions. These patterns of behaviour are considered in relation to various characteristics of the four fungi.

Rough bark diseases caused by *Caliciopsis* spp.

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Summary

The genus *Caliciopsis* is an ascomycete in the family *Coryneliaceae* and order *Coryneliales*. These fungi are characterized by a perithecium located on the top of a dark spine. Ascospores are produced in a more or less circular ascus with a very long narrow stalk. There are several species in this genus and its taxonomy has been studied in Canada by Funk. *Caliciopsis* develops mostly on the smooth bark of trees and invades the outer part of the cambium. The reaction of the host is a proliferation of corky masses that give a rough aspect to the smooth bark. The species *Caliciopsis pinea* is found mainly on white pine (*Pinus strobus*) in eastern North America. There is increasing interest in this pathogen because *C. pinea* is associated with top killing cankers of trees. We observed white pine samples collected in New Hampshire; the three bolts with visible cankers were associated with this top killing disease and show signs of *C. pinea*. Our observations show that 89% of fruiting bodies of *C. pinea* appeared on "healthy" bark; only 11% were seen on the margin or on the bark inside the canker margin. The symptoms of the cankers seen on sections of these logs are similar to *Cytospora* canker symptoms illustrated on spruce and very different from symptoms associated with *Caliciopsi pinea*. The presence of *C. pinea* on these cankers seems fortuitous and should not be considered as the causal agent of cankers. Isolation of microorganisms on the margin of these cankers should be performed to identify the causal organism that does not seem to produce fruiting bodies.

Pathological anatomy of Nectria canker on *Fraxinus mandshurica* var. *japonica* caused by *Nectria galligena* Bresadola

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Summary

The anatomical characteristics of Nectria canker on *Fraxinus mandshurica* var. *japonica* were presented. Typical cankers were conspicuous, round to oval, with uniform concentric rings of affected xylem in a target-like structure. Each concentric annual growth ring was wider than the corresponding annual rings lateral to the cankers. The xylem elements were extremely disoriented and the cambial zone became discontinuous and disappeared. Fewer and narrower vessel formations were observed, and water conduction took place only in the large vessels of the current year in the cankers. An inoculation test with the causal fungus produced similar anatomical abnormalities and revealed the process of canker formation.

Nectria galligena Bresadola によるヤチダモがんしゅ病の病態解剖

ヤチダモがんしゅ病の解剖学的特徴を明らかにした。典型的ながんしゅは円形ー楕円形で非常に目立ち、射撃の的のような同心円状の構造を呈していた。同心円を形成するそれぞれのリングは、その部分と対応するがんしゅ部横の年輪より、幅が広かった。木材要素の配列は、著しく乱れていた。形成層帯は不連続、あるいは消失していた。病原菌である *Nectria galligena*
の人工接種により、自然下のがんしゅと同様の解剖学的異常が再現され、がんしゅ形成過程も明らかになった。導管は口径が狭く、またその形成数も少なく、通水機能を有していたのは、当年性の大口径導管のみであった。

Key words: *Fraxinus mandshurica* var. *japonica*, *Nectria galligena*, canker, wood anatomy, water conductivity, inoculation

1 Introduction

Nectria canker, a serious perennial disease in broad-leaved trees, is caused by *Nectria galligena* Bres. In Hokkaido (a northern island of Japan), the canker on *Fraxinus mandshurica* var. *japonica* is widely distributed (SASAKI 1979). The infection causes twig blight, deformation of tree shape, and brittleness of the affected part. However, little attention has been given to anatomical aspects. To clarify these points, this manuscript deals with the precise anatomy of canker on *F. mandshurica*.

2 Materials and methods

2.1 Natural cankers

2.1.1 Sampling

Sampling of natural cankers was performed in the natural forests of Bifuka (northern Hokkaido) and Tomakomai (central Hokkaido).

2.1.2 Macroscopic and microscopic observations

The outside appearance and transverse and radial views of cankers were observed with the naked eye or under a dissecting microscope (magnification: x 8-40).

For general observations, samples of various sizes were fixed with FAA (formalin: acetic acid: 50% ethanol = 5: 5: 90) , divided into small pieces (approximately 1.5×1.5×1.5 cm) and then dehydrated in an ethanol series and embedded in cedukol (Merck). Transverse and radial sections 12 to 25 μm thick were cut on a sliding microtome. They were stained with 1% safranin O solution in 50% ethanol and 0.1% fast green solution in 95% ethanol, and were then observed under a light microscope.

For detailed observations of the cambial zone and the xylem and phloem, some samples were fixed with 4% glutaraldehyde solution in phosphate buffer and then embedded in epoxy resin. Transverse sections 1 μm thick were cut on an ultramicrotome (EM-ULTRACUT-J). They were stained with 0.3% safranin O aqueous solution and then observed under a light microscope.

2.1.3 Water conduction

The dye injection test was carried out to examine the water-conductive vessels in the branches with cankers in the naturally infected trees.

Two branches with several cankers were collected, and their freshly cut bases were soaked in a 0.2% safranin O aqueous solution for 90 minutes. After removal of the phloem, the area stained with the dye was examined with the naked eye. Transverse sections (12 μm thick) of the cankers and the lateral and opposite sides of the cankers were cut on a sliding microtone and observed under a light microscope.

2.2 Canker formation by inoculation

The inoculation test was performed on seedlings in July 1999. Holes were bored into the xylem with a cork-borer (4.5mm in diameter) on the stem of each seedling. A disc (4.5 mm in diameter) of potato dextrose agar (PDA: Eiken E-MF21) bearing mycelium of *N. galligena* cut from the margins of an actively growing plate culture was placed into holes. Control seedlings were made by the same method with PDA discs without mycelium. The samples were collected regularly from August 1999 to October 2001. The development of visible alterations, such as swelling, cracking and formation of cankers on the inoculated points, was observed with the naked eye or a dissecting microscope (magnification: x 8-40) during the course of the sampling. The samples were fixed, embedded, dissected (20 to 25 μm thick), double-stained and observed under a light microscope by the same method used in the case of the natural cankers.

2.3 Iodine stain

For the observation of starch granules in parenchyma cells in the xylem, the healthy and cankerous (both natural and inoculated) samples were stained with iodine and potassium iodine (MIYAZAKI et al. 2002) and immediately observed under a light microscope.

3 Results

3.1 Natural cankers

Typical cankers had a target shape due to the exposed uniform concentric rings of extremely deformed xylem (Fig. 1). On the transverse disks of the cankers, cambial cells already appeared to be dead, and the exposed xylem was discolored and decayed in many cases (Fig. 2). The phloem of the cankers was thick, and dead phloem could be broken off easily.

The affected xylem had much wider annual growth rings than the corresponding annual rings lateral to the cankers. Formation of each concentric ring of target-like structures was due to the continuous cambium necrosis (Fig. 2).

The wider annual rings were composed mainly of axial parenchyma cells and wood fibers and contained only a small number of vessels (Fig. 3). The diameter of the large vessels was narrower in the abnormal xylem area than in the healthy xylem. An irregular orientation of all xylem elements, such as ray and axial parenchyma cells, vessels and wood fibers, was commonly observed (Fig. 4).

The number of axial parenchyma cells stained with iodine in the affected xylem was obviously abundant compared to the healthy xylem (Fig. 5). There are four types of axial parenchyma cells in *F. mandshurica*: scanty paratracheal, vasicentric, terminal and diffuse (ISHIDA and OHTANI 1989). However, because the parenchyma was disordered in the affected xylem, the four types could no longer be distinguished.

In the cambial zone of the cankers and adjacent to that area, the arrangement of the cambial cells was disordered. Thick-walled mature cells were mingled with thin-walled immature cells (Fig. 6). Such cambial abnormalities were more obvious as the cankers developed further.

Anatomical abnormalities were also observed in the xylem lateral to the cankers. The width of the annual growth was narrower than that in the healthy xylem, and the small vessels were poorly developed.

In the affected phloem of the cankers, a large number of parenchyma cells and sclereids were commonly observed. It was very difficult to distinguish between ray parenchyma and axial parenchyma cells because the arrangement of the cells was extremely disordered. The sclereids were grouped in dense cluster (Fig. 7).

3.2 Water conduction

The conductive vessels, which were stained with the dye, bypassed the exposed (necrotic) xylem of the cankers. And the transverse sections showed that only the large vessels formed in the current year were stained in the cankers. In the xylem of the lateral and opposite sides of the cankers, the large vessels formed in the current year and the small vessels formed in the previous year and two years earlier were stained.

3.3 Canker formation by inoculation

Approximately one month after inoculation, swelling became obvious in the vicinity of the inoculation points. The outer bark became ruptured, and the inoculation points continued to swell every year. Most of the inoculation points became noticeable cankers with necrotic tissues.

One month after inoculation, active cell division with fewer vessels, were observed. Irregular growth of xylem continued every year. The irregular orientation of the xylem cells and the abundant axial parenchyma cells that were stained with iodine became obvious. Two years after inoculation, wider annual growth with fewer and smaller vessels (corresponding to concentric rings of target-like structures) was clearly observed (Fig. 8).

4 Discussion

Characteristic concentric rings of this canker seem to arise from yearly necrosis of phloem and cambial cells in the vicinity of the canker. As a result of the necrosis, the outer bark and phloem at the margins of the canker are removed, and one ring of affected xylem appears annually. Repetition of such yearly events results in the development of the concentric pattern of exposed xylem (Fig. 9).

The developmental process of this canker suggests that the ability of the pathogen to extend into the tissues is weak, although it can survive in a limited zone for a long period. Necrosis was restricted to tissues in the vicinity of the canker. No apparent anatomical abnormalities were found on the opposite side of the canker or on parts longitudinally distant from the canker. It is likely that the pathogen can live in phloem, xylem and cambial cells surrounding the canker.

The narrower annual rings on the lateral side of the cankers indicate that most of the photosynthate to produce new xylem cells was consumed on the canker side.

From the functional point of view, vessel formation is quite important. Fewer and narrower vessels result in greatly reduced water conductivity in the cankers because the theoretical conductivity in capillaries is proportional to the fourth power of their diameters (ZIMMERMANN 1983). Hence, the water conductivity of the cankers was greatly decreased. Although both the large and small vessels on the lateral and opposite sides of the cankers were conductive, the branches and stems with numerous or large cankers could not have conducted enough water at the beginning of the growth season until the large vessels of the current year had developed. The decrease in water conductivity in the cankers in early spring became one of the reasons for the debility or dieback of the seriously affected trees.

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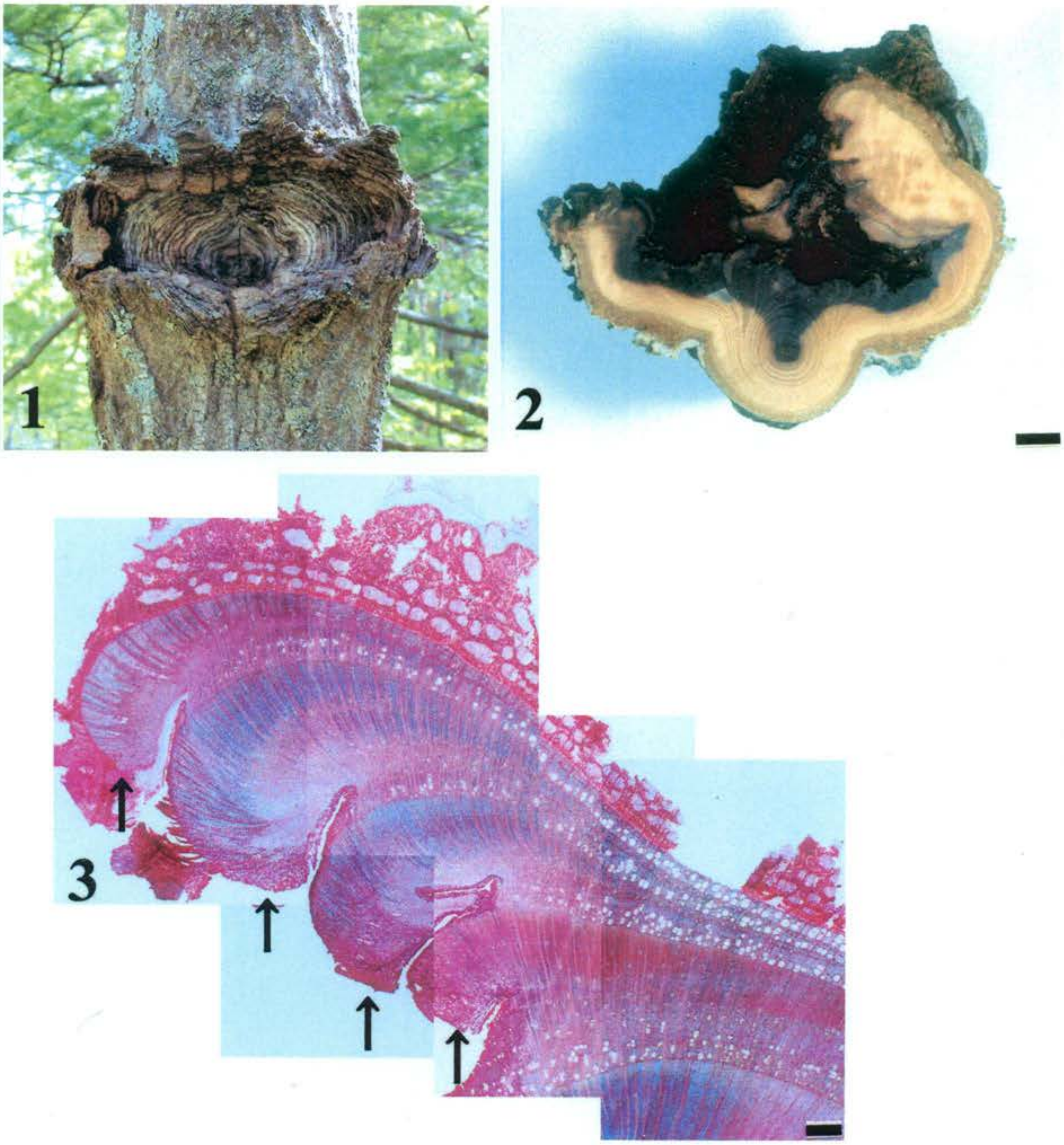
Figure legends

Figure 1. The target-like structure of a typical canker (photographed 29 May 2002 in its natural environment).
Figure 2. Transverse view of a typical canker. Scale bar = 1 cm.
Figure 3. Transverse view of a canker. Arrows indicate the concentric rings of the target-like structure. Scale bar = 500 μm.
Figure 4. Transverse view of the irregular orientation of the xylem elements of the canker. Scale bar = 500 μm.
Figure 5. Iodine stain of the cankers. Scale bar = 500 μm.
Figure 6. Cambial zone of the canker. Note the thick-walled mature cells (large arrows) and the thin-walled immature cells (small arrows). Scale bar = 50 μm.
Figure 7. Transverse view of the affected phloem. Arrows indicate the dense cluster of sclereids. Scale bar = 500 μm.
Figure 8. Transverse view of the canker approximately two years after inoculation. Arrows indicate the concentric rings of the target-like structure. Scale bar = 500 μm.
Figure 9. Diagrams of the formation of the target-like structure. A: Wide growth increments of xylem and phloem are formed simultaneously. B: The edges of the phloem become necrotic (arrows). C: The edges of the wide growth zones of xylem become exposed and visible (arrows). b = outer bark and phloem; p = pith.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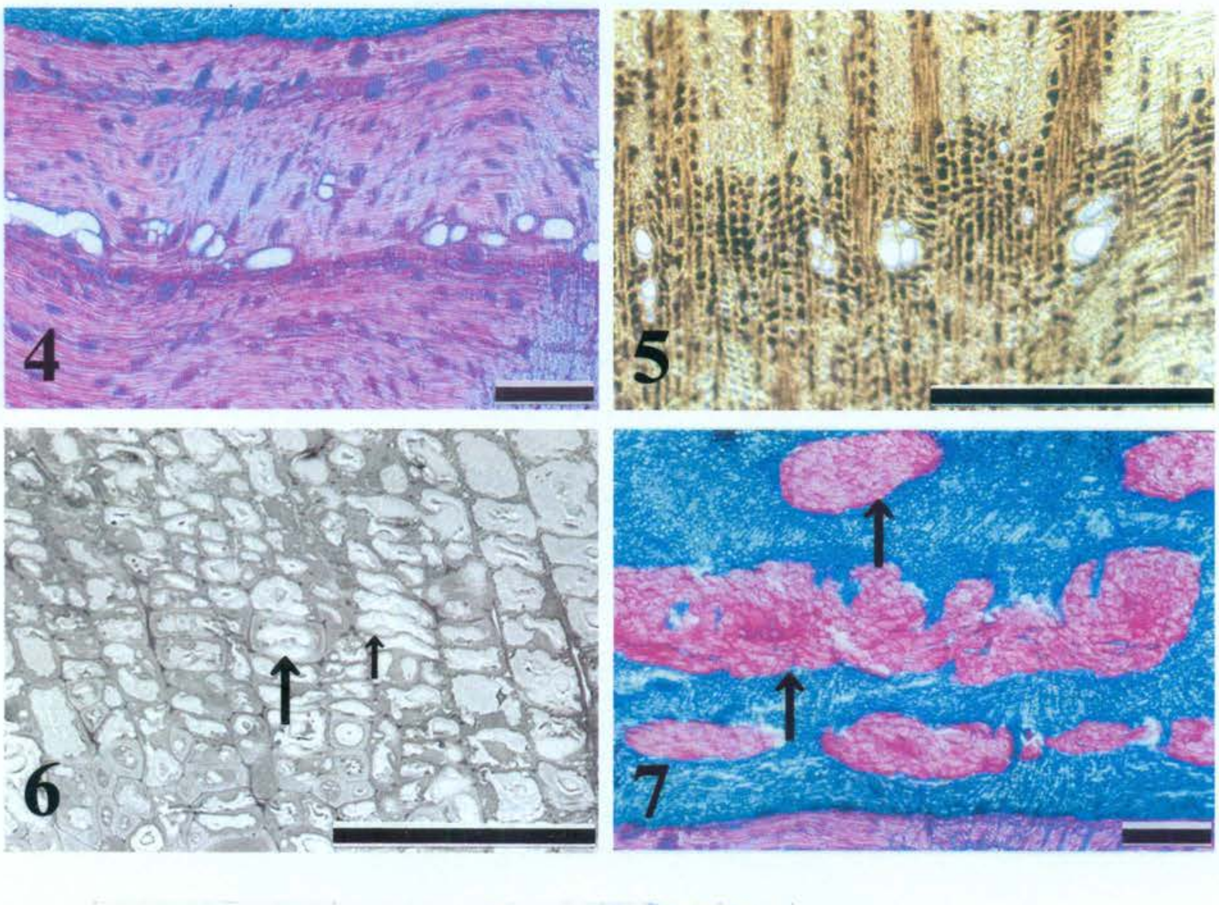
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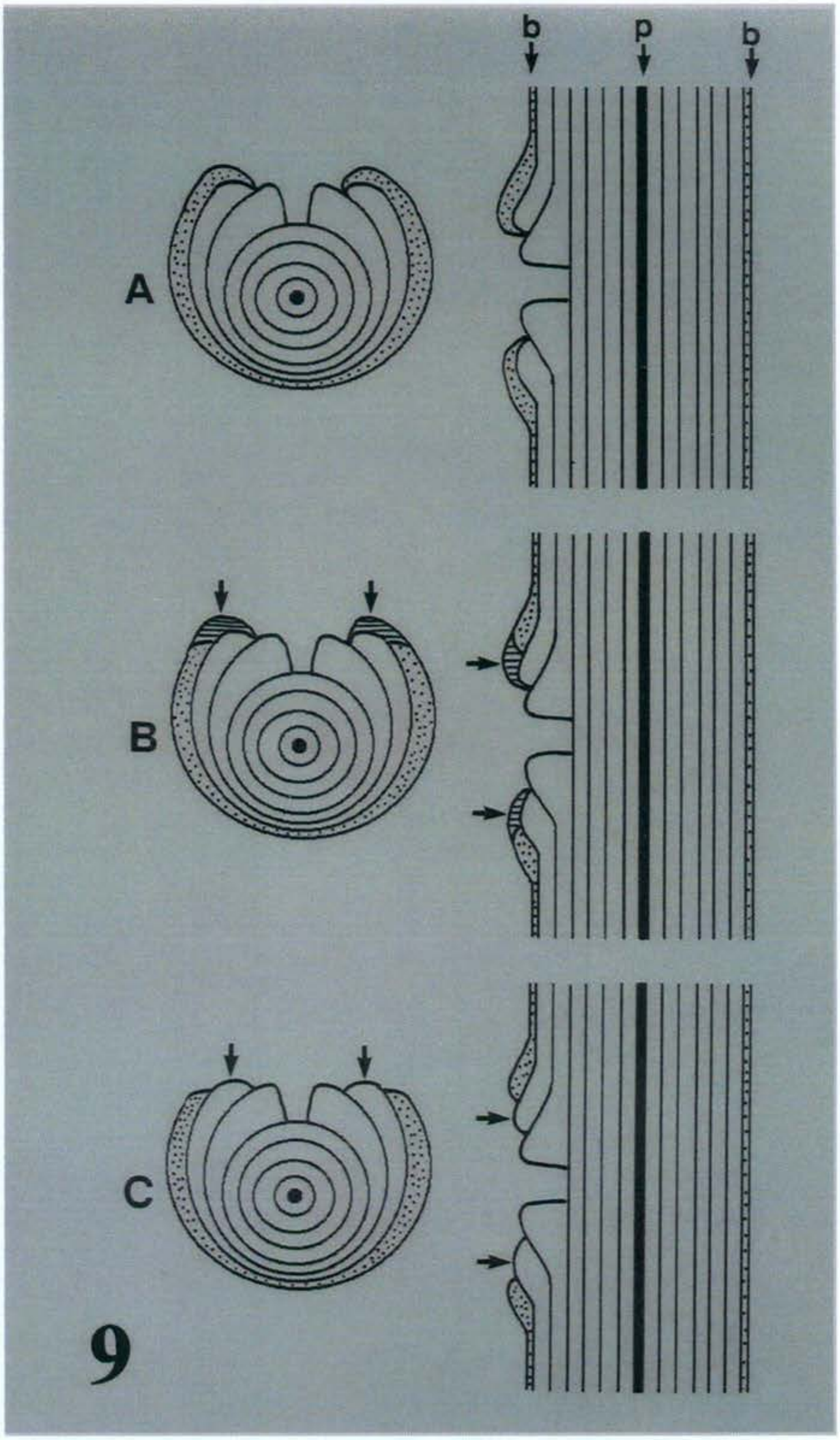
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Viability of conidia of Sirococcus clavigignenti-juglandacearum on exoskeletons of three coleopteran species

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Summary

Butternut canker, caused by the fungus *Sirococcus clavigignenti-juglandacearum*, is threatening survival of butternut (*Juglans cinerea*). Several insect species are known to carry conidia of the fungus. This study examines the vector potential of three coleopteran species, *Astylopsis macula*, *Eubulus parochus*, and *Glischrochilus sanguinolentus*. In the summers of 2001 and 2002, beetles were collected, rinsed, and artificially infested with conidia. The length of time beetles carried viable conidia (up to 16 days) and total numbers of conidia carried were determined. All three species carried viable conidia for up to 16 days. The maximum number of conidia carried by *A. macula*, *E. parochus*, and *G. sanguinolentus* at 0 hours was 14 million, 5 million, and 1 million, respectively. The mean range of conidia carried per beetle for the two years combined was 1.90 million at 0 hours to 7,105 at 16 days for *A. macula*; 1.95 million at 0 hours to 71 at 16 days for *E. parochus*; and 214,150 at 0 hours to 157 at 16 days for *G. sanguinolentus*. Artificially-infested and field-collected beetles were examined using scanning electron microscopy. Conidia were observed on the abdomen, thorax, and legs of artificially-infested individuals from all three species, and on the thorax and abdomen of field-collected *A. macula* and *E. parochus*. These data and observations suggest that all three species are potential vectors of conidia of *S. clavigignenti-juglandacearum*; however, *A. macula* and *E. parochus* may act as more effective vectors.

Magnetic resonance imaging of xylem dysfunction in *Quercus crispula* infected with a wilt pathogen, *Raffaelea quercivora*

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Summary

The large mortality rate of deciduous oaks, *Quercus serrata and Q. crispula*, has been a serious problem for the past two decades in Japan. The pathogen of this wilt disease, *Raffaelea quercivora*, enters the trunks by mass attacks by an ambrosia beetle, *Platypus quercivorus*. Physiological and cytological investigations of infected trees revealed that widespread discoloration and serious dysfunction had occurred in sapwood prior to the wilt. The present report discusses the mechanism of wilting based on the non-destructive observation of xylem dysfunction using the magnetic resonance (MR) imaging technique after the inoculation of three-year-old *Q. crispula* trees with the pathogen. The MR imaging shows water-containing tissues as high intensity as a result of the behavior of protons. Conductive vessels in healthy oak trees were recognized as whitish areas on the MR images. By one week after the inoculation with *R. quercivora*, the area approximately 1 cm above and below the inoculated sites showed low intensity and was looked darker. Eight weeks after the inoculation, the area of low intensity had reached about 2 cm above and below the infection site. Anatomical observations after the MR imaging revealed that water conduction had stopped and there was desiccation in the darker areas. T1 weighted images, which is used to detect the presence of fat or protein for medical purposes, showed the necrotic and discolored xylem area as high intensity. Some substances produced by the activity of the pathogenic fungus might be detected on T1 weighted images.

The growing (but not universal) problem of *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings in nurseries

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Summary

Sphaeropsis sapinea persists on or in stems of red pine (*Pinus resinosa*) nursery seedlings, and proliferates to cause collar rot and mortality after planting. In spring 2002, seven nurseries were surveyed to determine the potential range in frequency of asymptomatic persistence: three operated by the Wisconsin Department of Natural Resources (DNR), two by the Minnesota DNR, one by Michagan DNR, and one by USDA Forest Service (in Michigan). At each nursery five groups of 20 asymptomatic red pine seedlings were collected near an inoculum source (red pine windbreak), if present, and five groups of 20 asymptomatic seedlings were collected away from such a source. A segment of the lower stem/root collar from each seedling was surface-disinfested and incubated on tannic acid agar. Transfers were made from resulting colonies and the pathogen identified from pycnidia and conidia produced in culture. A subset of isolates was characterized using ISSR-PCR analysis. The pathogen commonly was identified from asymptomatic seedlings collected in all Wisconsin and Minnesota DNR nurseries, but was never detected from seedlings from the Michigan DNR or USDA nurseries (which lacked red pine windbreaks). Frequency of detection was greater (as high as 88%) from seedlings near windbreaks than from others. Most isolates were the A group of *S. sapinea*, but a few from one MN nursery were B group, now referred to as *Diplodia scrobiculata*. Persistence of *S. sapinea* on or in asymptomatic seedlings continues to be problematic, in terms of subsequent mortality and distribution of the pathogen.

Yellow-twig canker of pagoda dogwood

By R. W. Stack[1] and S. C. Redlin[2]

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Summary

Many plant-parasitic fungi have co-evolved with their respective hosts into a relationship called "balanced parasitism". We often explain it by citing extreme examples of "unbalanced parasitism", such as chestnut blight. Less extreme cases of unbalancing a more-or-less balanced relationship can be attributed to human activity. Examples might be fusiform rust in pine plantations or stem cankers in dense plantations of cottonwood and sycamore. The problems with those diseases arise out of intensive cultivation. The dogwoods with alternate leaves are few; their horizontal branching pattern is unusual and aesthetically pleasing. The ornamental dogwood *Cornus controversa*, native to Japan and China, is hardy only south of St. Louis, Missouri in the USA. The corresponding alternate-leaved native species, *C. alternifolia*, pagoda dogwood, is hardy to zone 3 and has a similar desirable branching habit. *Cornus alternifolia* occurs widely as a large shrub or small tree in the hardwood forest understory from northern Minnesota to eastern Canada and southward in the mountains to North Carolina. A fungal canker caused by *Cryptodiaporthe corni* affects *C. alternifolia* throughout its range. In the natural setting this canker would appear to be a good example of balanced parasitism. One conspicuous characteristic of this canker is the change of color of bark on infected twigs and branches. They turn a bright yellow-orange, hence the name "yellow twig". REDLIN and ROSSMAN (1991) describe the fungus in detail. Mature perithecia mostly appear in the bark on larger branches (>1.5 cm) while the conidial stage is present on nearly all infected tissue. Observations in native woodland suggest considerable variability in the host for response to the canker. In a casual stroll through the woods, one may see branch cankers which appear to be limited at the juncture of the main stem and one can often find some plants severely cankered while others are slightly or not at all affected. Those observations suggest genetic differences in susceptibility may be present. This plant is normally an understory species; when *C. alternifolia* is cultivated the problem of yellow twig canker is magnified, and may actually limit its use in the landscape. Two possible answers to this problem may be: 1) selection of naturally resistant individuals as parents for nursery production; 2) crossing of *C. alternifolia* with the apparently resistant *C. controversa*. REDLIN and STACK (2001) suggested the former based on observation of individual landscape plants. The potential of crossing *C. alternifolia* with *C. controversa* is not known. According to Donald Wyman of the Arnold Arboretum this fungus is not known to affect *C. controversa*.

References

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Beech bark disease in Canada

By G. LaFlamme

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Summary

In Canada, beech bark disease is a complex involving three organisms: the beech scale insect (*Cryptococcus fagisuga*), infesting and wounding the American beech (*Fagus grandifolia*), and pathogenic fungi of the genus *Nectria* that colonize these wounds (*N. coccinea* var. *faginata*, but also *N. galligena*). *Cryptococcus fagisuga* is an exotic insect that was introduced to Canada at Halifax, Nova Scotia from Europe around 1890. It was discovered there on European beech (*Fagus sylvatica*) seedlings planted in a public garden. Although *N. galligena* is native to North America, the fungus *N. coccinea* var. *faginata* was introduced; how, when, and where is uncertain. The disease causes deformation of the trunk and mortality of trees. While the area of affected forests continues to expand to the southwest in the range of American beech, not much research has been carried out in Canada. It is proposed to look into the effect of silvicultural practices on disease development. Biological controls of the insect and the pathogen have potential used in high value beech stands and also should be developed. Selection and reproduction of resistant beech must be accelerated. Finally, knowledge of the current distribution of the insect and the disease in eastern Canada should be updated.

Poster Presentations

Effectiveness of fungicides in protecting Douglas-fir shoots from infection by *Phytophthora ramorum*

By G. A. Chastagner[1], E. M. Hansen[2], K. L. Riley[1], and W. Sutton[2]

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Summary

The effectiveness of 20 fungicides in protecting Douglas-fir seedlings from infection by *Phytophthora ramorum* was determined. Some systemic products were applied about a week prior to bud break, while most treatments were applied just after bud break. One day after the post-bud break treatment applications, all the seedlings were inoculated and then incubated under optimal conditions for disease development. The only pre-bud break treatment that completely prevented infection was the drench application of Subdue MAXX. Pre-bud break drench applications of Stature, Insignia, and Terrazole had no affect on the number of infected seedlings. The reduction in infection by the pre-bud break applications of Heritage and Chipco Signature was variable and applications of Phostrol reduced infections by about 71 to 75%. Post-bud break applications of Dithane, Gavel, Maneb, and Polyram provided 100% control. Although not as consistently effective, applications of Champ Formula 2F, Reason, Daconil Ultrex, Stature, and IKF – 916, reduced the number of infected seedlings by 70 to 100%. Most of the other fungicides included in these tests provided more limited or variable reductions in the number of infected seedlings. These results indicate that several fungicides have the potential to provide excellent control of *P. ramorum* on Douglas-fir, including several common "protectant" types of fungicides.

Effectiveness of fungicides in controlling Rhabdocline needle cast on Douglas-fir Christmas trees grown in the Pacific Northwest

By G. A. Chastagner[1] and K. L. Riley

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Summary

Fungicides are often used in disease management programs to control development of Rhabdocline needle cast on Douglas-fir Christmas trees, particularly if highly susceptible intermountain provenances are being grown. Historically, applications of various formulations of chlorothalonil have been used to control this disease. Although this fungicide has consistently been one of the most effective materials available, there are a number of newer, environmentally friendly fungicides that growers are interested in using. During 2003-04, a test was conducted to compare the effectiveness of several newer fungicides with some that are commonly used to control needle cast. This test was conducted in a 9-year-old plantation of 21 different sources of Douglas-fir that are being tested for their susceptibility to needle cast caused by *Rhabdocline pseudotsugae* ssp. *pseudotsugae* at WSU Puyallup. Nine highly susceptible trees were selected for use in this test. Each tree was considered a block and the new growth on a single, randomly selected branch on each tree was sprayed to drip with each fungicide on May 8, 2003. An unsprayed branch on each tree served as the control. Treatment effectiveness was assessed on April 1, 2004, by rating the severity of needle cast on the 2003 needles on each branch on a scale of 0 (none) to 100 (severe needle cast on > 75% of the needles). The average disease rating for the unsprayed checks was 76.7. The level of disease on branches treated with Medallion (fludioxonil) or Switch (cyprodinil plus fludioxonil) was not significantly different from the checks. Although applications of Heritage (azoxystrobin) and Kocide (copper hydroxide) significantly reduced needle cast compared to the checks, these materials were not as effective as Insignia (pyraclostrobin), Daconil WeatherStik (chlorothalonil), Thiolux (sulfur), Fore (mancozeb) and Compass (trifloxystrobin). Data from this trial indicates that several fungicides have the potential to control Rhabdocline needle cast as effectively as chlorothalonil.

Climate indicators related to *Gremmeniella abietina* outbreak on Scots pine

By P. Hansson[1] and M. Ottosson Löfvenius[2]

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Summary

All tested climate indicators showed significant correlations for at least one season or type of pine forest. The results indicate that attacks by *G. abietina* are more related to the tested climate indicators for Scots pines in pure stands than for Scots pines in mixed stands.

1 Introduction

The aim of this study was to find climate indicators that correlate with large-scale disease occurrence of *Gremmeniella abietina* in *Pinus sylvestris* forests using standard data of temperature and precipitation from 1985–2000.

2 Materials and methods

Correlations between disease distribution 2002, collected by the National Forest Inventory, NFI and National Forest Disease Inventory, NFDI, (n=2,659), and five climate indicators describing the climate 1997–2000 were analyzed. Indicators were generated from 98 weather stations.

3 Results and discussion

The period preceding this largest known *Gremmeniella* outbreak in Sweden (Fig. 1) was characterized by a cold and wet growing season 1998, a mild and long intermediate period 1998/99 and an extremely wet growing season of 2000 (Fig. 2), which matches the two-year life cycle of *G. abietina*.

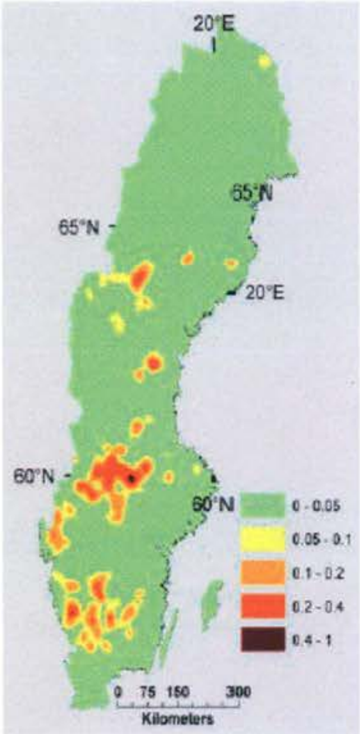

Figure 1. Proportions of pines on plots with >30% pine with Gremmeniella disease in 2002.

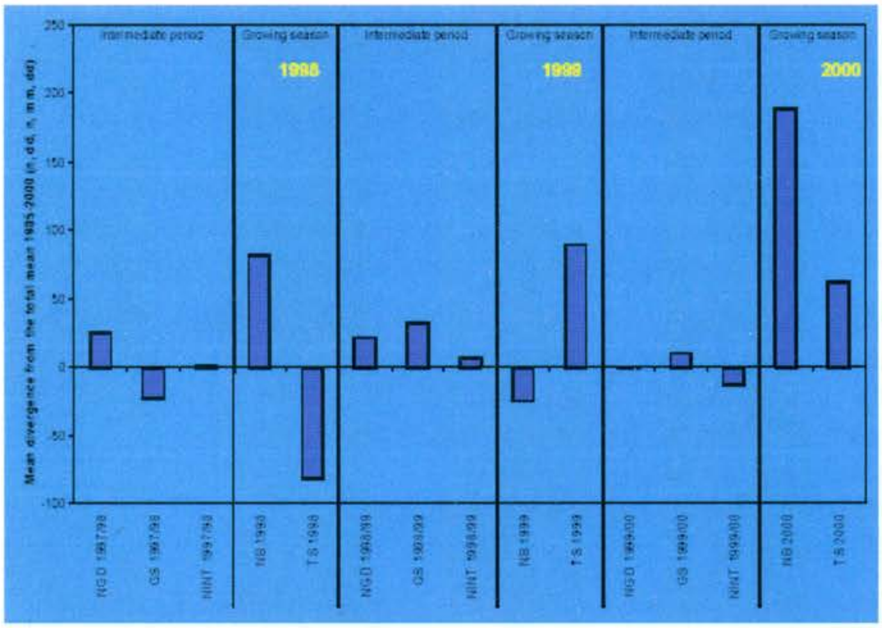

Figure 2. General pattern of the studied climate indicators, expressed as the mean divergence from the mean 1985-2000 for 50 standard weather stations.

	Mixed pine forest		Pine-dominated forest	
	Total	Severe	Total	Severe
NGD 1997/98	+	+	+	+
NGD 1998/99	-	-	-	-
NGD 1999/00	+	-	-	-
GS 1997/98	-	-	-	-
GS 1998/99	-	-	+	+
GS 1999/00	+	+	-	+
NINT 1997/98	-	-	+	+
NINT 1998/99	+	+	-	-
NINT 1999/00	-	-	+	+
NB 1998	-	-	-	-
NB 1999	+	+	+	+
NB 2000	+	+	+	+
TS 1998	-	-	+	+
TS 1999	-	-	-	-
TS 2000	+	+	-	-

Table 1. Directions of regression coefficients after GLM for "total" and "severe" *Gremmeniella* disease on NFI/NFDI plots with >30% (mixed) or >70% pine basal area. Yellow = significance level, $p < 0.05$, orange = $p < 0.01$ and red = $p < 0.001$.

Climate indicators:
NGD = number of days with daily temperature in the range – 6°C and + 5°C during the intermediate period;
GS = "*Gremmeniella* temperature sum" (dd = day degrees);
NINT = number of days between two growing seasons, the intermediate period;
NB = precipitation during the growing season (mm);
TS = temperature sum during the growing season, threshold +5°C (dd).

All tested climate indicators showed significant correlations for at least one season or type of pine forest (Table 1). The results indicate that attacks by *G. abietina* is more related to the tested climate indicators for Scots pines in pure stands than for Scots pines in mixed stands.

In relation to the general favorable weather sequence (Fig. 2), the disease incidence increased in areas that experienced the following sequence of weather anomalies:

1. relatively longer and colder intermediate period 1997/98,
2. warmer growing season 1998 and with lower precipitation,
3. shorter and colder intermediate period 1998/99,
4. wetter growing season 1999 also in mixed pine forests,
5. colder growing season 2000.

One plausible explanation for anomaly number 4 is that the spread of conidia could take place during the generally warm and dry growing season of 1999 in areas receiving comparably higher precipitation.

The effect of provenance on disease incidence of *Gremmeniella abietina* in 50-year-old *Pinus sylvestris*

By P. Hansson[1], J. Witzell[1], M. Wikström[1] and O. Rosvall[2]

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Summary

The severe outbreak of *Gremmeniella abietina* in Sweden during 2001 – 2003 mainly affected 30 – 60 year-old monocultures of *Pinus sylvestris* in three disease centres. The effect of latitudinal and altitudinal origin of *Pinus sylvestris* provenances on disease incidence of *Gremmeniella abietina* were evaluated in three 50-year-old provenance experiments situated in each of the three disease centres. The proportion of *Gremmeniella* affected crown was recorded in the autumn of 2001. In total 2,482 trees of 40 different provenances from latitude 59 to 68°N and from altitude 5 to 720 m a. s. l. were diagnosed. Seventy-six percent of the trees showed symptoms and the average proportion of affected crown in the whole material was 17%. The results showed that provenances transferred from south to north were more severely affected than provenances transferred to the south. No effect of altitudinal transfer was found. Five percent of the trees had more than 90% of the crown killed by *Gremmeniella*. Since the provenances probably had experienced the same spore pressure, the revealed differences indicate differences in susceptibility to *Gremmeniella abietina*.

1 Introduction

The severe outbreak of *Gremmeniella abietina* in Sweden during 2001 – 2003 mainly affected 30 – 60 year-old monocultures of *Pinus sylvestris* in three disease centers. The aim of this study was to evaluate the effect of latitudinal and altitudinal origin of *Pinus sylvestris* provenances on disease incidence of *Gremmeniella abietina.*

2 Materials and methods

The proportion of *Gremmeniella* affected crown was recorded in the autumn of 2001 in three 50-year-old Swedish provenance experiments with *Pinus sylvestris* (Table 1). In total 2,482 trees of 40 provenances from latitude 50 to 68°N and from altitude 5 to 720 m a. s. l. were diagnosed. Separate statistical analyses (GLM) were preformed for each experimental site. Independent variables were: latitude, altitude, and block. Similar analysis was done at tree level in order to test the effect of tree class (social rank).

Experimental site	Geographic location	Number of tested provenances	Latitudinal range of provenances (degrees N)	Altitudinal range of provenances (m. a. s. l.)
Björkvattnet, Sollefteå	63°26'N, 460 m.a.s.l.	21	58°54' – 68°04'	12 – 420
Högståsen Orsa	61°05'N, 365 m.a.s.l.	14	50°10' – 66°56	5 – 720
Eckersholm, Jönköping	57°35'N, 225 m.a.s.l.	7	56°18' – 62°03	5 – 385

Table 1. Description of experimental sites and tested provenance material.

3 Results and discussion

The results showed that 76 percent of the trees showed symptoms and the average proportion of affected crown in the whole material was 17%.

At Björkvattnet and Högståsen provenances transferred from south to north were significantly ($p<0.001$) more affected than provenances transferred to the south (Fig. 1).

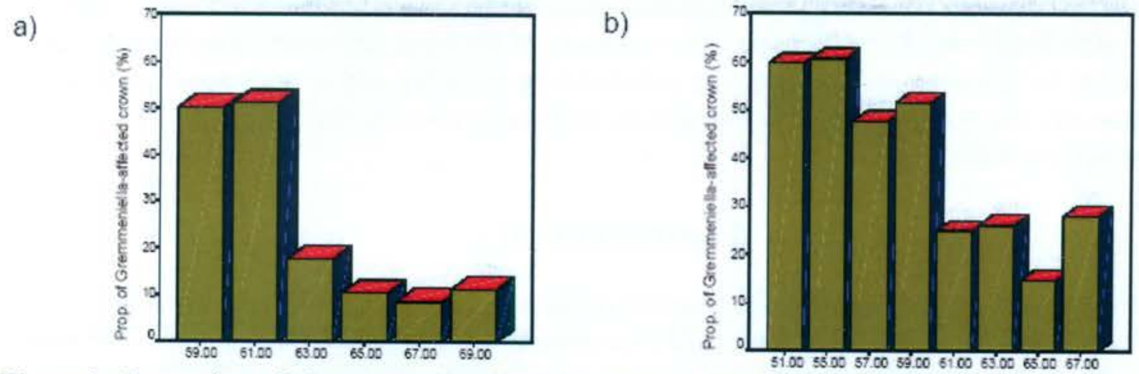

Figure 1. Proportion of *Gremmeniella*-affected crown vs. latitudinal origin of tested provenances at a) Björkvattnet (lat. 63), and b) Högståsen (lat. 61).

The effect of altitude of seed origin was significant only at Högståsen ($p=0.001$), where more severe disease was recorded for provenances from low altitudes. At this site provenances of both low and high altitude from about the same latitude were tested, facilitating analysis of altitudinal effects.

The proportion *Gremmeniella*-affected crown increased significantly ($p<0.001$) with decreasing social rank (or increasing suppression) within the stand (Fig. 2).

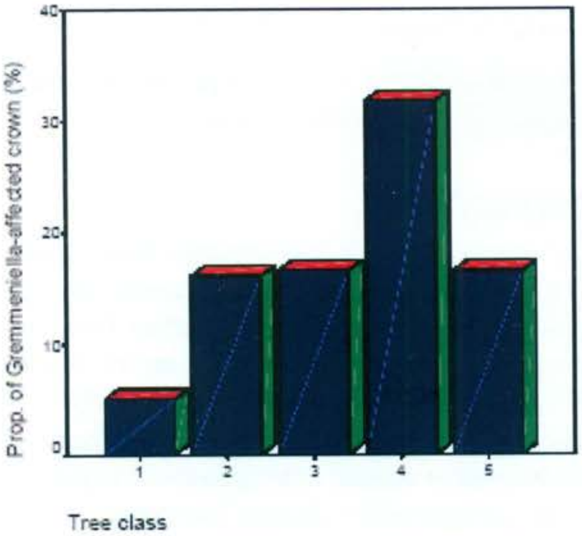

Figure 2. Proportion of *Gremmeniella*-affected crown vs. tree class (social rank) at all three sites (n=2470). 1=solitary trees, 2=dominant, 3=co-dominant, 4=suppressed, 5=heavily suppressed trees.

Five percent of the trees had more than 90% of the crown killed by *Gremmeniella*. Since the provenances probably had experienced the same spore pressure, the revealed differences indicate differences in susceptibility to *Gremmeniella abietina*.

The significance of incorrect provenance transfer for the large scale damages to commercial forests is yet to be investigated.

Characteristics of *Pinus sylvestris* stands attacked by *Gremmeniella abietina*

By P. Hansson and A. Frank

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Summary

During the latest epidemic in Sweden 2001-2003 more than 450,000 hectares forest land were affected by *Gremmeniella abietina*. This resulted in considerable economic losses. The aim of this study was to characterize the diseased stands using site variables from the stand data base of Holmen Skog AB to evaluate the effect of these variables on the disease incidence. We used all 670 reported diseased *Pinus sylvestris* stands (predominantly monocultures) owned by Holmen Skog in northern Sweden (approx. 61–65°N). These were compared with the same number of comparable reference stands. Using logistic regression we analyzed the effect of ten site variables on the *Gremmeniella* disease incidence. In total 4,989 hectares were classified as affected by *Gremmeniella* and therefore treated with sanitation actions. At the time of our study, 2,719 hectares were thinned and 2,279 hectares were clear felled. After our study a greater proportion was clear felled. Compared to the reference stands *Gremmeniella* affected sites were characterized by lower temperature sum, higher altitude, lower site index, poorer vegetation type and were classified as "spruce site" according to the National Forest Inventory. These site variables explained 80% of the variance in disease incidence. The results are in accordance with the general pattern of this epidemic – the disease centres were concentrated to former spruce sites on elevated areas, where the site fertility often is low and the climate harsh.

1 Introduction

The aim of this study was to characterize the diseased stands using site variables from the stand data base of the forest company Holmen Skog AB to evaluate the effect of these variables on the disease incidence. We used 670 *Pinus sylvestris* stands owned by Holmen Skog in northern Sweden (approx. 61–65°N) that were reported as diseased by *Gremmeniella* in 2001. These stands were compared with the same number of comparable "reference" stands. Using logistic regression we analyzed the effect of ten site variables on the *Gremmeniella* disease incidence.

Figure 1. A 10-year old severely affected monoculture of *Pinus sylvestris* planted on a former spruce site near Järvsö, north central Sweden. Photo: Per Hansson.

Figure 2. A severely affected *Pinus sylvestris* stand situated at an elevation in Värmland, central Sweden. Photo: Jesper Witzell.

2 Results and discussion

In total 4,989 hectares were classified as affected by *Gremmeniella* and therefore treated with sanitation actions. At the time of our study, 2,719 hectares were thinned and 2,279 hectares were clear felled. After our study a greater proportion was clear felled. The ten site variables explained 55% of the variance in disease incidence and altogether in a model predicted 80% of the observed cases correctly (Table 1). Compared to the reference stands *Gremmeniella* affected sites were characterized by:

- lower temperature sum;
- higher altitude (Fig. 3);
- lower site index;
- poorer vegetation type;
- "spruce site" according to the National Forest Inventory.

The results are in accordance with the general pattern of this epidemic – the disease centers were concentrated to former spruce sites on elevated areas, where the site fertility often is low and the climate harsh.

Variable	Coeff.	Wald	Sign.
Site index	0.160	14.868	<0.001
Sloping	0.143	1.728	0.189
Vegetation type	-0.187	26.367	<0.001
Soil texture	-0.304	2.232	0.135
Soil moisture	-0.187	0.390	0.532
Temperature sum	-0.007	46.803	<0.001
Altitude	0.015	177.064	<0.001
Exposition	-0.042	1.403	0.236
Stand area	-0.004	0.083	0.774
Spruce site	0.776	10.547	0.001

Table 1. Regression coefficients and significance levels for tested variables.

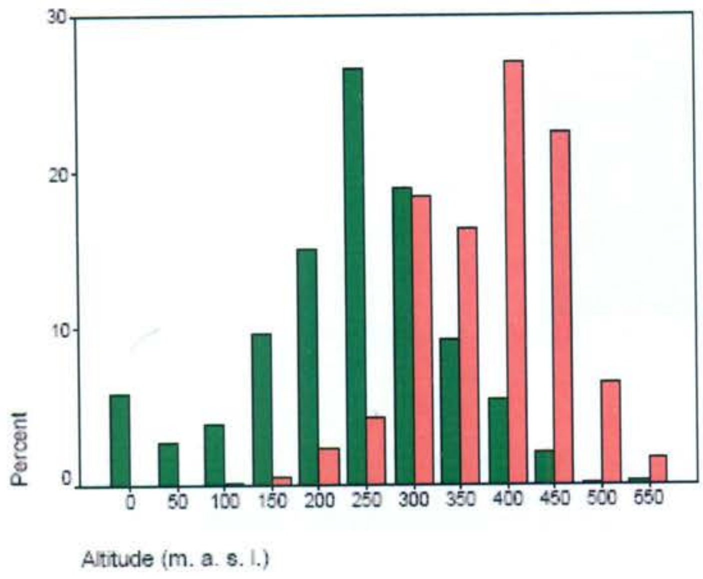

Figure 3. Proportion (%) of Gremmeniella-affected *Pinus sylvestris* stands (red) and reference stands (green) at different altitudes.

An estimation of economical loss due the *Gremmeniella abietina* outbreak in Sweden 2001 – 2003

By P. Hansson[1], M. Persson[1] and H. Ekvall[2]

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Summary

The aim of this study was to estimate the economical loss in *Pinus sylvestris* stands treated with sanitation thinning or clear-felled due to attack by *Gremmeniella abietina*. The study was based on stand and management data from two forest companies, Stora Enso and Holmen Skog AB. Calculations were made on 233 stands consisting of 93 clear-felled stands and 140 stands treated with sanitation thinning. The total area was 2,045 hectares and mean age of the stands was 44 years. When carrying out calculations the business economics calculation program Plan33 was used. It is a computer program created at SLU-Umeå for planning and analysis of forest companies. Plan33 values stands from the point of view of business economics. It enables most accurate calculation of yield value which can be utilized to maximize and optimize the present value of the company. The results pointed to a mean monetary loss of 780 USD/hectare when clear-felling was required and to 340 USD/hectare when sanitation thinning was needed amounted. In total for the 233 studied stands, the monetary loss was calculated to about 1 million USD. Using stepwise multiple regression analysis a simple formula was created which can be used for calculating the monetary loss in an individual stand attacked by *Gremmeniella*. The entire area in Sweden attacked by *Gremmeniella* during 2001 - 2003 has been estimated to approximately 450,000 hectares. Including extra costs associated with *Gremmeniella* attacks the monetary loss for attacked stands in whole Sweden may reach up to 250 million USD, depending on how complete the sanitation actions will end up. Monetary losses due to the reduced growth are not included in these calculations.

1 Introduction

The aim of this study was to estimate the economical loss in *Pinus sylvestris* stands treated with sanitation thinning or clear-felling due to attack by *Gremmeniella abietina.*

2 Materials and methods

The study was based on stand and management data from two Swedish forest companies, Stora Enso and Holmen Skog AB (PERSSON 2003). Calculations were made on 233 stands consisting of 93 clear-felled stands and 140 stands treated with sanitation thinning. The total area was 2,045 hectares and mean age of the stands was 44 years. When carrying out calculations the business economics calculation program Plan33 was used. It is a computer program created at SLU-Umeå for planning and analysis of forest companies.

Example of clear felled *Gremmeniella*-affected *Pinus sylvestris* stand near Järvsö, northern central Sweden, August 2001. Photo: Per Hansson.

A sanitation causing a monetary loss of about 780 USD/hectare due to harvest of a productive middle-aged stand! Photo: Per Hansson.

3 Results and Discussion

The results pointed to a mean monetary loss of 780 USD/hectare when clear-felling was required and to 340 USD/hectare when sanitation thinning was needed amounted. In total for the 233 studied stands, the monetary loss was calculated to about 1 million US

Using stepwise multiple regression analysis a simple formula was created which can be used for calculating the monetary loss in an individual stand attacked by *Gremmeniella*:

$$C = 5{,}647.9 - 8.8 \times \text{Age} + 16.8 \times \text{Volume} - 38.5 \times \text{Area}$$

If the stand will be thinned instead of clear felled: –3,685.4 SEK
If the stand is situated in Northern Sweden: –1,771.9 SEK
SEK = Swedish Crowns

The results showed that the monetary loss increased with increasing standing volume in the affected stand (Fig. 1).

The entire area in Sweden attacked by *Gremmeniella* during 2001 - 2003 has been estimated to approximately 450,000 hectares (WULFF et al in preparation). Including extra costs associated with *Gremmeniella* attacks, the monetary loss for attacked stands in Sweden may reach up to 250 million USD, depending on how complete the sanitation actions will end up. Monetary losses due to the reduced growth are not included in these calculations.

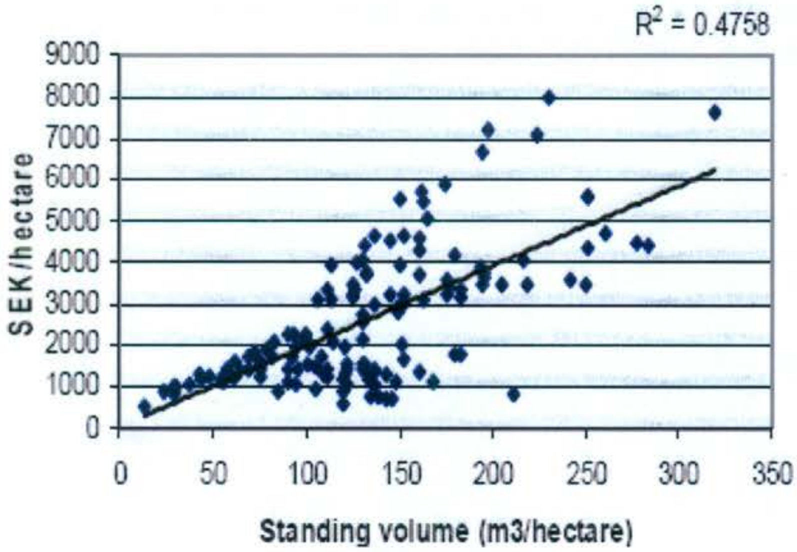

Figure 1. Relation between monetary loss and standing volume (from PERSSON 2003).

Reference

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Fungi associated with fresh fallen leaves of *Taxodium mucronatum* in Nuevo Leon, Mexico

By J. G. Marmolejo

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Summary

For this study fresh fallen leaves of *Taxodium mucronatum* were sampled in March 2002, March 2003, and March 2004 in 20 localities in Nuevo Leon, Northeast Mexico. Leaves were kept under moist conditions during two weeks. After this time leaves were examined under a microscope for fungi. The results of this study showed well-defined pattern of fungi. Thirteen species were identified. Those were: *Alternaria alternata* (Fr.) Keissl., *Cercosporidium sequoiae* (Ellis & Everh.) Baker & Partridge, *Chaetospermum chaetosporum* (Pat.) A.L. Sm. & Ramsb., *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Discosia fraxinea* (Schwein.) Di Cosmo, *Dycima pulvinata* (Berk. & M.A. Curtis) Arx, *Hymenoscyphus* sp., *Mycosphaerella* sp., *Wiesneriomyces laurinus* (Tassi) P.M. Kirk, *Periconia byssoides* Pers., *Pestalotiopsis funerea* (Desmasz.) Steayert, *Phyllosticta* sp., and *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis.

An anatomical technique for serial sectioning of plant tissues using cellulose tape

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Summary

A quite unique and useful technique for serial sectioning of plant tissues using cellulose tape was presented. In this article, we demonstrate the serial sectioning of various tree tissues such as the initial symptoms of the canker on *Acer japonica* (pathogen: unknown), the buds of *Picea jezonensis* attacked by *Adelges japonicus* Monzen (aphid) and the roots of *Lalix gmelinii* with Hartig net, by this method.

Key words: Serial sectioning, plant tissues, cellulose tape, microscopic observations

1 Introduction

Generally, when researchers made serial sectioning of plant tissues, they always embedded the specimens in paraffin, and then made them into "series of a ribbon of paraffin sheets" (SASS 1958). However, if the specimens were too hard or brittle, the specimens would be wrinkled or shattered by the sectioning of the microtome blade. To avoid this, some researchers have used cellulose tape (BONGA 1961; PALMGREN 1954; SAHO 1974). However, they have not described the detailed anatomical procedures. Hence, I modified the technique in more appropriate way for serial sectioning.

2 Materials and methods

Plant materials were fixed and embedded in paraffin by the normal technique (SASS 1958). The surface of the paraffin specimen was shaved by the microtome blade, a piece of cellulose tape was attached to the paraffin surface (Fig. 1), and then the sections were made (Fig. 2). These sections with tape were serially attached to glass slide (glass slide 1) by Haupt's adhesive (1% gelatin and 2% phenol in SAHO 1974) (Fig. 3), the sections were covered by another glass slide (glass slide 2), were then pressed together by a metal clip (Fig. 4). These pressed slides were heated (approximately 70 ºC) for about one hour and were then dried for one day. After that, the attached slides were soaked in xylene for one day (Fig. 5). The adhesive of the cellulose tape and the paraffin were dissolved by xylene, after which, slide 2 and the cellulose tapes were easily removed from slide 1. So, we can get the slides with the serial sectioning of the specimens. These slides with the serial sectioning can be stained by many kinds of staining materials. The stained sections were embedded in Canada balsam (Fig. 6), and were then ready for microscopic observations.

3 Result and discussion

By using this method, the serial sectioning of quite fragile plant tissues such as the fine roots of *Larix gmelinii* with ectomycorrhizae was done successfully (PROKUSHKIN et al. 2002). In the case of hard plant tissues such as tree xylem, if the size of the specimens are small (approximately within 10 mm in diameter), this method is also useful.

It is better to attach cellulose tape on slide 2 (without adhesive) before covering slide 1 (Fig. 3), because the tape on slide 2 makes a little space between two slides, hence xylene will easily get into the space between the two slides.

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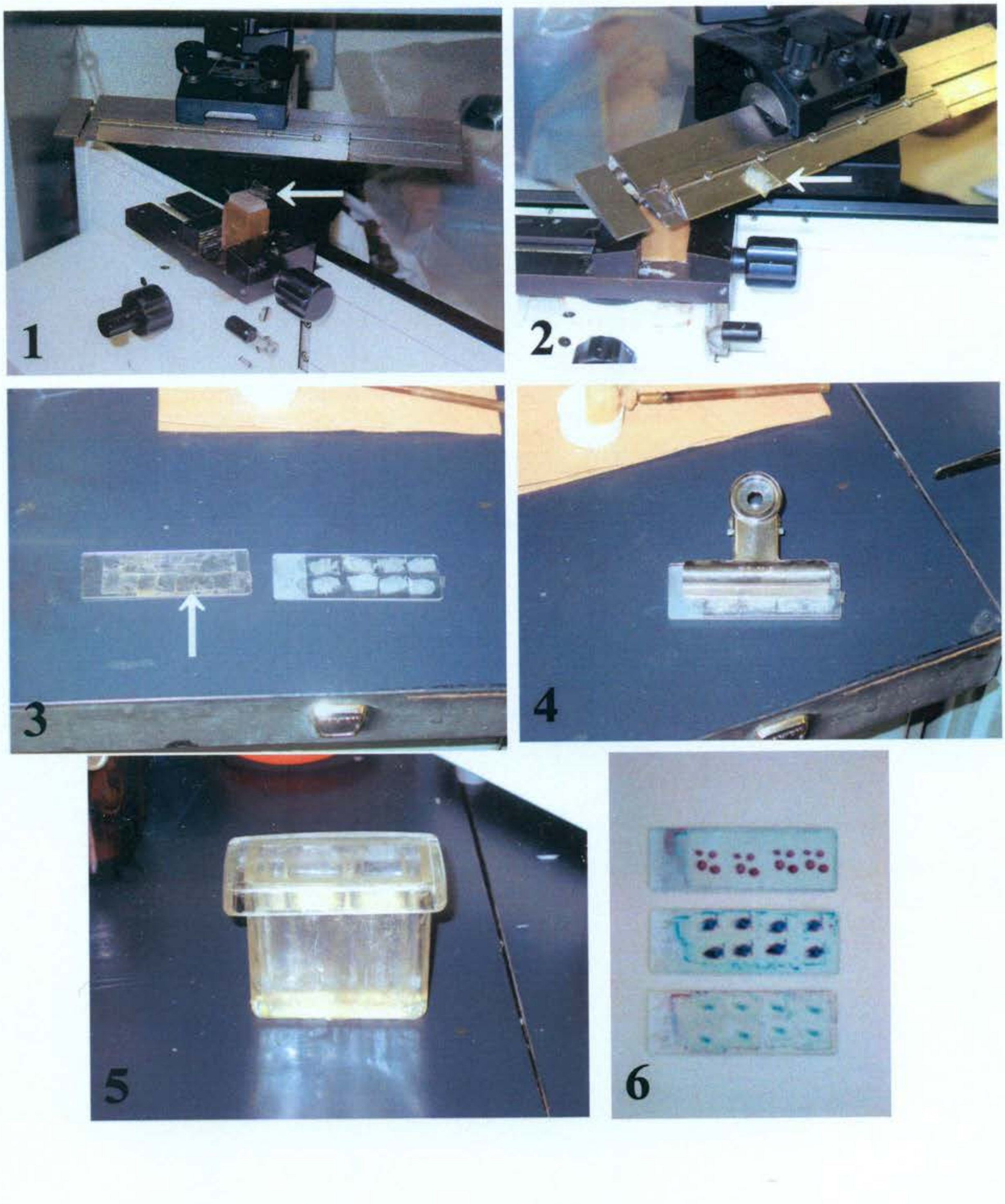
Figure legend

Figures 1-6 The sectioning procedures.
Figure 1. Note the piece of tape on the paraffin block (arrow).
Figure 2. Note the section with tape on the blade (arrow).
Figure 3. The sections were serially attached on the slides. Arrow indicates the tape on slide 2.
Figure 4. The slides were pressed together by a metal clip.
Figure 5. The slides were soaked in xylene for one day.
Figure 6. The stained preparations.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Risk from *Sirococcus conigenus* to understory red pine seedlings

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Summary

Changes (for aesthetic reasons) in red pine (*Pinus resinosa*) management practices include creation of irregular, longer edges of harvest sites and leaving residual overstory trees, instead of complete clearcut harvesting before replanting. Also, in a departure from traditional even-aged plantations in which trees of one age are grown, there is increasing interest in development of uneven-aged or multicohort red pine stands, in which trees of several ages would grow together. To confirm the risk of damage from the shoot blight pathogen *Sirococcus conigenus* (which inhabits crowns of overstory trees) to regeneration in these situations, red pine seedlings were planted in ten 50-seedling plots in the understory of a maturing red pine stand in northcentral Wisconsin in spring 2002 and 2003. Initial seedling establishment and symptom development was recorded during each growing season and in fall of each year seedlings were harvested to determine the presence of the pathogen. By fall 2002 and 2003, respectively, mean shoot blight incidence on established seedlings was 89% and 98%. Most of these seedlings were dying or dead and pycnidia with conidia of *S. conigenus* were present on almost all of the seedlings. The shoot blight pathogen *Sphaeropsis sapinea* also was detected, though much less frequently. These results support previous research and concern about the risk that shoot blight pathogens pose to understory red pine seedlings.

A test of the validity of screening poplar clones for long-term canker disease damage by responses to inoculation with *Septoria musiva*

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Summary

The potential to predict long-term canker disease damage to poplars by responses to inoculation with *Septoria musiva* has been demonstrated, but screening is not yet routine. To test validity of screening procedures, 15 clones of commercial interest to a forest industry cooperator (including resistant and susceptible standards) were inoculated during their first season of growth in the field. On each stem, the fourth or fifth fully expanded leaf was removed and an agar plug colonized by an aggressive isolate of *S. musiva* was placed over the resulting wound. Four months after inoculation, canker incidence, canker length, and percent of stem circumference affected (girdle) were recorded. Resulting cankers resembled those attributed to *S. musiva* and responses of standards were consistent with those previously reported. Clones varied greatly in canker incidence (17-96%), mean canker length (5-55 mm), and mean girdle (10-91%). Logistic regression analysis was used to compare responses with canker disease damage categories assigned on the basis of information from longer-term field studies. Incidence, canker length, and girdle data all were informative, and for most clones there was a high probability that responses to inoculation correctly predicted assigned canker disease damage categories. These results validate previous work indicating the feasibility and benefit of screening juvenile poplar clones for responses to inoculation with *S. musiva* before extensive field trials and release to growers.

Significance of the iodine reaction in *Rhabdocline*, *Sarcotrochila*, and *Hemiphacidium*

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Summary

The presence or absence of an iodine reactive ascus tip has been applied inconsistently in Hemiphacidiaceae. The genera *Hemiphacidium* (I-) and *Sarcotrochila* (I+) have been differentiated based on the reaction despite a high degree of morphological and ecological similarity, while in the genus *Rhabdocline*, the iodine reaction has been used to differentiate two pathogenic species and circumscribe subspecies. Comparison of the internal transcribed spacer region of nuclear ribosomal DNA for species in these genera reveals less than 2% sequence divergence between *Hemiphacidium longisporum* and *Sarchotrochila macrospora*, a value that is comparable to the level of divergence between sister species in *Rhabdocline*. This result is consistent with the high degree of morphological similarity between *H. longisporum* and *Sarchotrochila macrospora*, both of which are endophytes of *Pinus contorta*. Molecular phylogenetic analyses indicate that currently recognized subspecies of *R. weirii* are non-monophyletic, and that currently recognized subspecies of *R. pseudotsugae* form distinct sister lineages. The polyphyly of *R. weirii* suggests that the presence or absence of an I+ or I- ascus pore is not a good predictor of species circumscriptions. To de-emphasize the importance of the iodine reaction in classification within Hemiphacidiaceae and to promulgate a classification more consistent with relationships revealed by molecular phylogenetics, we propose that *Hemiphacidium* be synonymized under *Sarcotrochila* and that subspecies of *Rhabdocline pseudotsugae* and *R. weirii* be raised to species rank.

New disease on English yew (*Taxus baccata*) in Norway

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Summary

The fungus *Cryptocline taxicola* was found for the first time in Norway on severely damaged English yew (*Taxus baccata*) in the summer of 2001. The damage might partly be due to the mild fall in 2000, followed by a sudden drop in temperature before the plants had hardened. New samples taken in the fall of 2003, showed that *C. taxicola* was still present, but did not cause as much damage as in 2001. The fungus attacks and kills the current season needles and shoots. This is also reported from Germany, USA, and Canada on *Taxus* spp. Acervuli form on both the upper and lower side of the needles, and the epidermis ruptures as they break through. Acervuli are visible on green needles. As the disease develops the needles and whole shoots turn brown. From acervuli on needles incubated in saturated air at room temperature, a cream coloured spore mass oozes out. On dry needles acervuli appear dark and a bit sunken. In May 2004, a pathogenicity test was started.

1 Introduction

Symptoms of *Cryptocline taxicola* were observed on a severely damaged hedge of English yew (*Taxus baccata*) in the summer of 2001 at the campus of the Agricultural University of Norway (at Ås, south eastern Norway). The damage had been noticed for a few years prior to the first identification in 2001, but not as severe as it appeared that year. Similar symptoms on *T. baccata* were also observed in Lyngdal at the southern coast of Norway in 1999, but the fungus was never confirmed present. The symptoms found were as follows (TALGØ et al. 2003): Acervuli formed on both the upper and lower side of green needles and shoots, and the epidermis ruptured as they broke through. The acervuli were visible to the naked eye, but a good stereo microscope was needed to see the acervuli properly. As the disease developed, the needles and whole shoots turned brown. On dry needles acervuli appeared dark and a bit sunken.

Recently *C. taxicola* has been reported on *T. baccata* from Germany (WULF and PEHL 2001; PEHL and WULF 2001) and on *T. brevifolia* from USA (Vermont) and Canada (Quebec) (VUJANOVIC and ST. ARNAUD 2001). The symptoms are identical to the symptoms observed in Norway. From Germany it is said that further investigations will take place to establish wether the disease is more harmful than previously thought. From Canada it is believed that inappropriate stand selection, unfavourable humid conditions and a thin organic soil layer may predispose *T. brevifolia* to infection by *C. taxicola*.

2 Materials and methods

2.1 Samples

In 2001, samples from the *T. baccata* hedge at Ås were analysed at the Norwegian Crop Research Institute. The samples were incubated in saturated air at room temperature. New samples were incubated in the fall of 2003, and isolations were carried out on potato dextrose agar (PDA).

2.2 Pathogenicity test

To fulfil Koch's postulate a pathogenicity test was carried out to confirm the parasitic ability of the isolated fungus. One ml of sterile, distilled water was added to a sporulating PDA-culture (90 mm Petri dish) of *C. taxicola*. Three different cultivars of *Taxus baccata* were inoculated by dipping a cotton tipped sterile applicator in the spore suspension and carefully applying it to the soft, new shoots. The cultivars were 'Summergold', 'Melfard', and 'Schwarzgrün'. Some plants were wounded by applying Carborundum prior to the inoculation. The room temperature was kept at 20°C and the plants were given 16 hours daylight. The test started on 22 May 2004. A polyethylene bag was kept over each plant until 7 June. After the bags were removed, the relative humidity was kept at approximately 80 %.

3 Results and discussion

3.1 Samples

Three days after incubation of the plant material, a cream coloured spore mass oozed out from the acervuli (Fig. 1). The acervuli measured ±0.3 mm in diameter. Glass slides for microscope examination were prepared and spore sizes were measured. The spores were hyaline, nearly egg shaped and measured 11-18 x 5-8 µm. On PDA, the colonies were nearly black on the reverse side of the Petri dish, but on the upper surface some aerial mycelium gave the culture a greyish appearance. The culture was slow growing on PDA. At 21°C and no light the culture grew 1.3 mm per day. The fungus fit the description of *C. taxicola* by PETRAK (1925).

Figure 1. Spores (conidia) of *Cryptocline taxicola* oozing out from acervuli on the upper side of a needle from an infected *Taxus baccata* hedge. Ås, Norway, 2003. Photo: E. Fløistad.

3.2 Pathogenicity test (Koch's postulate)

By 9 August 2004, visible symptoms were only traced on 'Summergold' that had been wounded by Carborundum. A few needles had turned brown and acervuli developed. As may be seen from the picture, remains of the Carborundum still stuck to the needle (Fig. 2).

The results from the inoculation test did not indicate that *C. taxicola* was very aggressive. The severe damage in 2001 might partly be due to the mild fall in 2000, followed by a sudden drop in temperature before the plants had hardened.

Figure 2. On this needle from *Taxus baccata* 'Summergold', acervuli developed after inoculation by *Cryptocline taxicola.* The needle was wounded by applying Carborundum prior to inoculation, and remains of it can be seen between the acervuli. On dry needles like this one, acervuli appear dark, but the older acervuli towards the base of this needle are even darker than normal, because *Cladosporium* sp. had established secondarily. Photo: E. Fløistad.

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Coastal Oregon Field Trip

Meeting of the International Union of Forestry Research Organizations (IUFRO)
Working Party 7.02.02, Foliage, Shoot, and Stem Diseases

Tuesday, 15 June 2004

8:00 Leave Hilton Garden Inn, Corvallis

8:15 See Diplodia on pine at 9th St.-Hwy 99W junction in Corvallis

8:30 Leave Diplodia site

9:15 See poplar diseases near Salt Creek Rd.

9:45 Leave poplar site

10:45 Stop at store in Beaver

11:00 Leave store

11:15 See Swiss needle cast growth affects near Bixby Rd.

12:00 Leave SNC site

12:15 See SNC affects on old-growth Douglas-fir at top of hill

12:30 Lunch

1:00 See SNC and spruce diseases at overlook

1:30 Leave overlook

2:00 See SNC-fungicide-treated plots near E. Beaver Rd.

2:30 Leave fungicide site

3:15 Arrive in Pacific City to see ocean

4:00 Leave Pacific City

5:15-5:30 Arrive at Hilton Garden Inn in Corvallis

Central Oregon Field Trip

Meeting of the International Union of Forestry Research Organizations (IUFRO)
Working Party 7.02.02, Foliage, Shoot, and Stem Diseases

Thursday, 17 June 2004

8:00	Leave Hilton Garden Inn, Corvallis
9:10	Arrive Cascadia State Park, see diseases on ash, hemlock, yew, and rhododendron
9:40	Leave Cascadia
10:20	Arrive Lost Prairie, rest stop, see diseases on true fir, white pine, and other conifers
10:50	Leave Lost Prairie
11:15	Arrive Mt. Washington overlook, see Cache Mt. fire
11:30	Leave Mt. Washington overlook
11:50	Arrive at Camp Sherman store, feed trout in famous Metolius River
12:05	Leave Camp Sherman
12:15	Arrive at Head of the Metolius, lunchstop, see disease in ponderosa pine
1:15	Leave Head of the Metolius
1:45	Arrive at Toll Station site (421 Rd. jct.), see diseases in true fir, lodgepole pine, and ponderosa pine
2:15	Leave Toll Station site
2:35	Arrive at McKenzie Hwy
3:00	Arrive at McKenzie Pass, weather and snow permitting
6:00	Arrive in Corvallis via Eugene
	or
2:45	Arrive in Sisters, OR
3:30	Leave Sisters
6:00	Arrive in Corvallis via Santiam Pass

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